UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE ÓPTICA Y OPTOMETRÍA

TESIS DOCTORAL

Evaluación de la estructura de las glándulas de meibomio mediante tecnología infrarroja

Infrared imaging of the meibomian gland structure

MEMORIA PARA OPTAR AL GRADO DE DOCTORA

PRESENTADA POR

Laura Rico del Viejo

DIRECTORES

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DOCTORAL THESIS

INFRARED IMAGING OF THE MEIBOMIAN GLAND STRUCTURE

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Laura Rico del Viejo

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European Commission

"I am among those who think that science has great beauty. A scientist in his laboratory is not only a technician, he is also a child place before natural phenomenon, which impress him like a fairy tale."

***Marie Skłodowska Curie**, Nobel Prize in Physics (1903) and Chemistry (1911).*

"Science and everyday life cannot and should not be separated"

***Rosalind Franklin**, Nobel Prize in Chemistry (1982).*

"For a research worker the unforgotten moments of his life are those rare ones which come after years of plodding work, when the veil over natures secret seems suddenly to lift and when what was dark and chaotic appears in a clear and beautiful light and pattern."
***Gerty Cori**, Nobel Prize in in Physiology or Medicine (1947).*

"The first telescope opened the heavens; the first microscope opened the world of the microbes; radioisotopic methodology, as examplified by RIA [radioimmunoassay], has shown the potential for opening new vistas in science and medicine"

***Rosalyn Yalow**, Nobel Prize in Physiology or Medicine (1977).*

"Young people, especially young women, often ask me for advice. Here it is, valeat quantum. Do not undertake a scientific career in quest of fame or money. There are easier and better ways to reach them. Undertake it only if nothing else will satisfy you; for nothing else is probably what you will receive. Your reward will be the widening of the horizon as you climb. And if you achieve that reward you will ask no other."

***Cecilia Payne-Gaposchkin**, Henry Norris Russell Lectureship of the American Astronomical Society (1976).*

"It is imperfection - not perfection - that is the end result of the program written into that formidably complex engine that is the human brain, and of the influences exerted upon us by the environment and whoever takes care of us during the long years of our physical, psychological and intellectual development."

***Rita Levi-Montalcini**, Nobel Prize in Medicine (1986).*

"Different and diverse points of view make science stronger"

***Donna Strickland**, Nobel Prize in Physics (2018).*

First quote included in the previous page belongs to Marie Skłodowska Curie - a researcher who gives her name to the prestigious scholarship that I have been able to enjoy. I felt that I need to include a bit of her knowledge in this work. She has been an example for me and was one out of very few women researchers that are recognized worldwide and throughout history excelled in science, technology, mathematics or engineering, leaving an important legacy. Many of them have remained in shade and battled against people who did not allow them to freely access education, to publish their research, people who prohibited them from obtaining funding or even those that allowed them to work in precarious conditions and without any support, because they were women. They had to fight against stereotypes to develop their research career. They have also contributed to numerous and important advances in science and their names are not well known. For this reason, this research work is especially dedicated to THEM, TO ALL THOSE WOMEN RESEARCHERS. Also, to those that will come, courageous and determined to take their rightful place in science and society. ***FOR WOMEN IN SCIENCE!***

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ABSTRACT

Introduction

Dry eye disease (DED) is a multifactorial disease of the ocular surface considered one of the most frequently encountered ocular conditions seen by eye care practitioners. Nowadays, DED is estimated to affect between $5 - 50\%$ of the worldwide population. Furthermore, the prevalence of DED increases linearly with age which makes DED a growing public health concern as the global population of older people is expected to be more than double its current amount by 2050. According to DED classification, the aqueous deficient dry eye (ADDE) and evaporative dry eye (EDE) are the two major DED types and are considered to exist on a continuum rather than as separate entities. Despite this, according to the current DED understanding, an evaporative component is more common than an ADDE component. Currently, the Meibomian gland dysfunction (MGD) is considered the leading cause of EDE. This condition may result in alteration of the tear film, symptoms of eye irritation, clinically apparent inflammation and ocular surface disease. Therefore, any change that occurs in the morphology of the MG or in its secretion has an important clinical impact.

Recently, the non-contact infrared meibography (NIM) has become the most widely used tool for both researchers and clinicians for the assessment of the MG structure. Many studies have confirmed the use of this technology for diagnosis and management of MGD. Indeed, NIM allows the detection of MG abnormalities such as meibomian gland loss (MGL), shortening, dilation and distortion. The MGL or *dropout* refers to the partial or total loss of acinar tissue and it is one of the most common MG features reported in the literature. Currently, there is no gold standard in the classification of MG assessed by NIM but most of them are based on the MGL. Previous studies found significant correlations between MGL and some tear film parameters (such as tear film break-up time (TBUT), non-invasive break-up time (NIBUT), lipid layer thickness (LLT), Schirmer test, MG expressibility and corneal staining) and subjective symptomatology (Ocular surface disease index questionnaire (OSDI) and McMonnies questionnaire), suggesting its possible diagnostic value. However, other studies

concluded that asses the MGL alone as clinical parameter has not enough DED diagnostic value, and it should be interpreted jointly with other clinical parameters.

Additionally, the multifactorial nature of the DED makes difficult for eye care practitioners to carry out a correct diagnosis and monitoring. For this reason, more objective, less invasive and more repeatable methods and technologies have been developed in order to obtain more valuable information for DED diagnosis.

This dissertation is an in-depth work focused on the study of the MG structure revealed by NIM. The main research goals of this work were: to assess the effect of ageing on the ocular surface parameters since it has been reported to be a risk and relevant factor for MG and the ocular surface. Also, to study the relationship between the MGL revealed by NIM and the ocular surface parameters in order to update MGD classification based on the MG structure. In addition, to study the relationship between new objective MG morphology parameters and the ocular surface parameters. Finally, to study and compare the thermal characteristics of DED and healthy subjects using infrared thermography.

Material and Methods

In order to accomplish these objectives, a clinical protocol was designed that included a complete assessment of the ocular surface with classical tests both invasive and non-invasive, as well as some new technology for the ocular surface assessment. The following measurements were included: a clinical anamnesis; tear film osmolarity by TearLab Osmolarity System; automated measurements with the Keratograph 5M (K5M); ocular surface temperature (OST) using a non-contact infrared thermography camera; a slit lamp biomicroscope with $\times 10$ magnification, cobalt blue illumination, a Wratten 12 yellow-barrier filter and fluorescein sodium ophthalmic sterile strips were used to observe ocular surface staining and TBUT. Lissamine Green strips were used to assess lid wiper and the conjunctival staining. Additionally, an assessment of the eyelid was carried out. Furthermore, a meibography image of each eyelid was obtained using the K5M. Finally, the Schirmer test was performed with topical anaesthesia with the eye closed and after 5 minutes the length of the wetting was measured.

Results

This experimental work is organized into four independent studies. In the first study the clinical protocol explained above was applied monocularly to a total of 110 participants (mean age; 44 ± 19 years, 70 females and 40 males) in order to study the first objective of this work. The main results of this study showed elderly population present ocular surface changes when compare to a young population. Although the majority of the ocular surface parameters studied presented a fair correlation with age, these results give us relevant information of the ageing of the ocular surface and how it could affect the DED diagnosis. Additionally, females from this study showed more changes due to ageing than males who presented better ocular surface conditions than those of their age- matched female group.

In the second study a total of 161 participants were included (mean age; 42 ± 17 years, 91 females and 70 males) whom were grouped according to the MGL graded using the subjective scale *meiboscore* introduced by Reiko Arita et al.2008. These groups were compared and the relationships between MGL and several ocular surface parameters were evaluated. In addition, age was included as covariant in the relationship between MGL and the ocular surface parameters since the mean age of the participants in this study was higher in those groups with higher MGL. The findings of this study suggest that a MGL higher than 50% is accompanied by signs of increased osmolarity, redness and staining of the ocular surface. Despite this, when age was included as a covariant only the corneal staining was correlated with MGL, emphasizing the influence of ageing on the MG morphology and also on several ocular surface parameters. Overall, these findings suggest that age-matched groups should be compared in order to know the contribution of the MGL on the ocular surface as well as establish a valid cut-off values for DED diagnosis.

The third study of this doctoral thesis consisted of the use of an automated algorithm that analyses infrared images of the MG using image processing techniques (*developed by the Department of Biomedical Engineering, Wroclaw University of Science and Technology, Wroclaw, Poland*) to obtain objective information about the MG morphology. Several objective MG parameters such as the MGL, gland length, gland width and the gland irregularity (also named as tortuosity or MG distortion) were

extracted of a total of 149 meibography images from the everted upper eyelids (UL) from the second study of this work. The main results of this study showed that the majority of the objective MG morphology parameter, which were obtained with the automated algorithm (Objective MGL, length and width of the gland) are highly influenced by age. Therefore, their influence on the ocular surface should be assessed by comparing age-matched groups as previously described in the second study. Regarding MG irregularity, its role as a clinical parameter is not clear according to the findings of this study. Instead, it could be a prodromal sign of gland loss. Therefore, as long as the gland is not atrophied it will be functioning and the ocular surface will not be affected.

The latest study involved the assessment of the OST of 86 participants (48 healthy and 38 DED eyes, mean age; 39 $\pm$ 12 and 49 $\pm$ 19 years, respectively) using an infrared thermography camera. The OST of both groups was registered for 40 seconds, allowing subjects to blink naturally. Several OST metrics were proposed in order to study the OST changes during the recording, assessing the first and last complete interblink interval (IBI). The main results of this work showed slightly thermal differences between DED and healthy eyes as well as ADDE and EDE. These differences were not statistically significant when the complete thermal recording was assessed. Indeed, this could be explained since the OST could be regulated by the warming effect provided by the eyelid in each blink. These findings show that the evaluation of the thermal behaviour during the IBI could provide useful information about the thermal changes of the ocular surface in DED and healthy eyes.

Conclusions

Summarizing, this research work provides a new knowledge about the MG morphology and its relationship with several important ocular surface parameters. This could help eye care professional to interpret and carry out a better the DED diagnosis. At the same time, the clinical utility of the infrared thermography to study the tear film dynamics has been used for the study of the tear film dynamics, especially in DED.

RESUMEN

Introducción

El ojo seco o síndrome de ojo seco (SOS) es una enfermedad multifactorial de la superficie ocular considerada como una de las afecciones oculares más frecuentes observadas por los profesionales de la visión en la práctica clínica. Hoy en día, se estima que el SOS afecta al 5-50% de la población mundial. Además, su prevalencia aumenta linealmente con la edad, lo que hace que el SOS sea un problema de salud pública creciente, ya que se espera un aumento del doble de la población mundial de personas ancianas para 2050. Según la clasificación del SOS, se pueden distinguir principalmente dos tipos: el ojo seco acuodeficiente, caracterizado por un déficit en la capa acuosa de la película lagrimal y el ojo seco evaporativo, el cual se caracteriza por una alteración en la evaporación de esta. A pesar de esta diferenciación, recientemente se ha observado que ambos tipos de SOS pueden coexistir simultáneamente, aunque el componente evaporativo tiende a ser el más común. Actualmente, la disfunción de las glándulas de meibomio (DGM) se considera la causa principal de la SOS evaporativo. Esta condición puede provocar alteración de la película lagrimal, síntomas de irritación ocular, inflamación clínicamente aparente y enfermedad de la superficie ocular. Por lo tanto, cualquier cambio que se produzca en la morfología o en la secreción de las glándulas puede tener un impacto clínico importante.

Actualmente, la meibografía infrarroja se ha convertido en la herramienta más utilizada por investigadores y por clínicos para la evaluación *in vivo* de la estructura de las glándulas de meibomio. Muchos estudios han confirmado el uso de esta tecnología para el diagnóstico y manejo de la DGM. De hecho, la meibografía infrarroja permite la detección y evaluación de diferentes anomalías de las glándulas de meibomio, como la pérdida del tejido glandular (o también comúnmente denominado como *dropout*), el acortamiento, dilatación y la tortuosidad de estas. Concretamente, el *dropout* se refiere a la pérdida parcial o total del tejido glandular y es una de las características morfológicas más evaluada en la literatura científica relacionada con el diagnóstico de

SOS y DGM. En la actualidad, no existe un método de cuantificación de referencia y/o clasificación del *dropout* basada en la meibografía infrarroja, pudiendo realizarse de forma subjetiva utilizando diferentes escalas, semiautomáticamente con la ayuda de un software para delimitar el área deseada o en algunos casos, de forma automática mediante softwares basados en análisis y procesado de imagen. Estudios previos han encontrado que el *dropout* se correlaciona de forma significativa con algunos parámetros de la película lagrimal como el tiempo de ruptura de la película lagrimal invasiva y no invasiva, el espesor de la capa lipídica, el test de Schirmer, la expresividad de las glándulas, la tinción corneal e incluso con la sintomatología subjetiva (cuestionarios comúnmente utilizados como el Ocular surface disease index (OSDI) y el cuestionario McMonnies), lo que sugiere su posible valor diagnóstico. Sin embargo, otros estudios concluyen que el área de *dropout* solo como parámetro clínico no tiene suficiente valor diagnóstico y debe interpretarse junto con otros parámetros clínicos. Además, la naturaleza multifactorial del SOS dificulta a los profesionales de la visión realizar un correcto diagnóstico y manejo de esta condición. Por esta razón, se han desarrollado nuevos métodos y tecnologías más objetivas, menos invasivas y más repetibles para obtener información válida para el diagnóstico de SOS.

Este trabajo de investigación esta centrado en el estudio de la estructura de las glándulas de meibomio mediante la utilización de meibografía infrarroja. Los principales objetivos son los siguientes: el primer objetivo pasa por evaluar el efecto del envejecimiento en los parámetros de la superficie ocular, ya que se ha reportado que es un factor relevante tanto para las glándulas de meibomio como para la superficie ocular. El segundo objetivo consiste en estudiar la relación entre el área de *dropout (*observada mediante meibografía infrarroja y cuantificada subjetivamente) y los parámetros de la superficie ocular. El tercer objetivo consiste en estudiar la relación entre los nuevos parámetros morfológicos de las glándulas de meibomio (como el área de *dropout* objetivo, longitud, anchura e irregularidad de la glándula) y los parámetros de la superficie ocular. Finalmente, el cuarto objetivo consiste en estudiar y comparar las características térmicas de ojos sanos y ojos que sufren de SOS mediante el uso de termografía infrarroja.

Material y Métodos

Para llevar a cabo estos objetivos, se diseñó un protocolo clínico que incluía una evaluación completa de la superficie ocular y sus componentes, incluyendo pruebas de diagnóstico clásicas y también nuevas tecnologías para la evaluación de la superficie ocular tanto invasivas como no invasivas. Se procedió a realizar las siguientes medidas (de menos invasivo a más invasivo): elaboración y análisis de la historia clínica del participante; osmolaridad de la película lagrimal medida con el dispositivo TearLab; medidas no invasivas de la superficie ocular con el dispositivo Keratograph 5M (K5M); temperatura de la superficie ocular (TSO) utilizando una cámara termográfica; evaluación completa de los diferentes componentes de la superficie ocular mediante biomicroscopía. Para ello, la lámpara fue configurada con un aumento de $\times$10 con luz difusa para una observación general y aumentos más altos para observar con más detalle partes como por ejemplo el borde del párpado y los orificios glandulares. También se utilizó la iluminación azul cobalto, filtro amarillo Wratten 12 y tiras de fluoresceína y verde lisamina para observar la integridad de la córnea, conjuntiva, párpados y la estabilidad de la película lagrimal. Posteriormente a esta evaluación, se obtuvo una imagen de meibografía de cada párpado utilizando la cámara infrarroja incorporada en el K5M. Finalmente, la prueba de Schirmer se realizó con anestesia tópica durante 5 minutos con el ojo cerrado.

Resultados

Este trabajo experimental está organizado en cuatro estudios de investigación independientes. Para llevar a cabo el primer objetivo, el protocolo clínico mencionado anteriormente fue aplicado monocularmente a un total de 110 participantes (edad media; 44 $\pm$ 19 años, 70 mujeres y 40 hombres). Los principales resultados de este estudio mostraron que la población anciana presenta cambios en la superficie ocular en comparación con una población joven. Aunque la mayoría de los parámetros de la superficie ocular estudiados presentaron una justa correlación con la edad, estos resultados nos brindan información relevante sobre el envejecimiento de la superficie ocular y cómo podría afectar al diagnóstico de SOS. Además, las mujeres de este estudio

mostraron más cambios debido al envejecimiento en comparación a los hombres, los cuales presentaron una mejor condición de la superficie ocular.

En el segundo estudio se incluyeron un total de 161 participantes (edad media; 42±17 años, 91 mujeres y 70 hombres) que se agruparon en cinco grupos según el área de *dropout* mostrado (*total meiboscore*) de acuerdo con la escala de cuantificación subjetiva introducida por Reiko Arita y colaboradores en 2008. Estos grupos se compararon y se evaluó la relación entre el área de *dropout* y varios parámetros relevantes de la superficie ocular. Posteriormente, la edad se incluyó como covariante en esta relación ya que la edad media de los participantes de este estudio fue mayor en aquellos grupos con mayor área de *dropout*. Los hallazgos de este estudio sugieren que un área de *dropout* superior al 50% se acompaña de signos en la superficie ocular, como por ejemplo un incremento en la osmolaridad, enrojecimiento ocular y tinción corneal y conjuntival. A pesar de ello, cuando la edad fue incluida como covariante, solo la tinción corneal se correlacionó con el área de *dropout*, enfatizando la influencia del envejecimiento en la morfología de las glándulas de meibomio, pero también en varios parámetros de la superficie ocular. En general, estos hallazgos sugieren que para conocer la contribución o influencia del área de *dropout* como parámetro para el diagnóstico de SOS es necesario la comparación entre grupos de la misma edad para poder establecer valores de corte válidos para el diagnóstico.

Para el tercer estudio de esta tesis doctoral, fue utilizado un nuevo algoritmo basado en procesado de imagen (desarrollado por el *Departamento de Ingeniería Biomédica, Wroclaw University of Science and Technology, Wroclaw (Polonia))* que analiza de forma automática las meibografías para obtener información objetiva sobre la morfología de las glándulas de meibomio. Se extrajeron varios parámetros objetivos de la glándula como el área de *dropout* objetivo, la longitud, la anchura y la irregularidad de la glándula (también llamada tortuosidad o distorsión) de un total de 149 meibografías del párpado superior obtenidas previamente. Los principales resultados de este estudio mostraron que la mayoría de los parámetros morfológicos objetivos de las glándulas de meibomio obtenidos con el algoritmo están también influenciados por la edad y, por tanto, su influencia en la superficie ocular debe evaluarse comparando grupos de la misma edad tal como se ha mencionado previamente en el segundo estudio. En relación con la irregularidad de la glándula como parámetro, este estudio sugiere que podría

representar la etapa prodrómica antes de perder la glándula. Por lo tanto, significa que mientras la glándula esté todavía presente, esta seguirá funcionando y solo en presencia de una gran cantidad de *dropout*, la superficie ocular se verá realmente afectada.

El último estudio de esta tesis se llevó a cabo la evaluación de la TSO de 86 participantes (48 ojos sanos y 38 SOS, edad media; 39±12 y 49±19 años, respectivamente) utilizando una cámara termográfica. La TSO de ambos grupos se registró durante 40 segundos mientras los participantes parpadeaban de forma natural mirando a la cámara. Varias métricas de temperatura fueron extraídas para estudiar los cambios de la TSO durante los 40 segundos completos de grabación térmica pero también en el primer y último intervalo completo de parpadeo. Los principales resultados de este trabajo mostraron que los ojos con SOS y los ojos sanos, así como los participantes con ojo seco acuodeficientes y evaporativo presentaban ligeras diferencias térmicas, aunque estas no fueron estadísticamente significativas. Esto podría deberse a la regulación térmica ejercida por el párpado en cada parpadeo. A la luz de estos hallazgos, la evaluación del comportamiento térmico durante el intervalo de parpadeo podría proporcionar información útil sobre los cambios térmicos de la superficie ocular en SOS y en ojos sanos.

Conclusiones

En resumen, este trabajo de investigación proporciona nuevos conocimientos sobre la morfología de las glándulas de meibomio y su relación con parámetros relevantes de la superficie ocular que podrían ayudar al profesional de la visión a interpretar y llevar a cabo un mejor diagnóstico del SOS. Al mismo tiempo, en este estudio se ha demostrado la utilidad clínica de la termografía infrarroja para el estudio de la dinámica de la película lagrimal y en especial en esta condición multifactorial.

ABSTRAKT

Zespół suchego oka (ZSO) jest wieloczynnikowym schorzeniem powierzchni ocznej i jedną z najczęściej obserwowanych w praktyce klinicznej dolegliwości okulistycznych. Obecnie szacuje się, że ZSO dotyka od 5 do 50% światowej populacji. Ponadto, zachorowalność na ZSO wzrasta liniowo z wiekiem. Szacuje się, iż populacja osób starszych ulegnie do 2050 roku podwojeniu, przez co ZSO może stać się znaczącym problem zdrowia publicznego. Zgodnie z przyjętą ogólnie klasyfikacją, podtyp ZSO związany z niedostatecznym wydzielaniem warstwy wodnej filmu łzowego (ang. aqueous deficient) oraz podtyp związany z nadmiernym jego parowaniem (ang. evaporative) są dwoma najbardziej powszechnymi rodzajami ZSO, które nierzadko są współistniejące. Badania naukowe pokazują, że ZSO spowodowane zwiększonym parowaniem łez występuje częściej niż pozostałe typy. Obecnie uważa się, że za ten podtyp odpowiada w dużej mierze dysfunkcja gruczołów Meiboma (ang. Meibomian gland dysfunction, MGD). Dysfunkcja ta powoduje zaburzenia filmu łzowego, podrażnienie, stan zapalny i chorobę powierzchni oka. Stąd też, każda zmiana zachodząca w morfologii gruczołów Meiboma lub w ich zdolności wydzielniczej ma duże znaczenie kliniczne.

Bezkontaktowa meibografia w świetle podczerwonym (ang. non-contact infrared meibography, NIM) jest najczęściej używaną techniką oceny i wizualizacji gruczołów Meiboma. Badania naukowe potwierdzają przydatność tej metody w diagnostyce i ocenie postępów terapii MGD. NIM pozwala na wykrywanie anomalii gruczołów Meiboma tj. ubytki (ang. Meibomian gland loss, MGL), ich skrócenie, rozszerzenie lub zniekształcenie. MGL spowodowane jest zanikiem (częściowym lub całkowitym) tkanki gruczołowej i jest najczęściej raportowaną w literaturze zmianą zachodzącą w tych gruczołach. Obecnie nie ma ustandaryzowanej metody klasyfikacji MG przy pomocy NIM, jednakże większość prezentowanych w literaturze skali opiera się na parametrze MGL. Poprzednie badania wykazały znaczące korelacje między MGL i parametrami określającymi jakość filmu łzowego, tj. czas przerwania filmu łzowego mierzony nieinwazyjnie i z użyciem fluoresceiny, grubość warstwy lipidowej filmu łzowego, wynik testu Schirmera, wysięk z gruczołów Meiboma, czy uszkodzenie nabłonka rogówki, a także między subiektywnymi wrażeniami pacjentów (mierzonymi przy pomocy

kwestionariuszy OSDI lub McMonnies'a). Wszystkie te korelacje sugerują diagnostyczną wartość parametru MGL. Mimo to, badania naukowe pokazują, iż samo MGL nie wystarczy jako kliniczny marker ZSO i musi być zawsze interpretowane w oparciu o inne zmienne. Dodatkowo, wieloczynnikowa natura ZSO utrudnia specjalistom ochrony wzroku szybką i poprawną diagnostykę i monitorowanie przebiegu choroby. Z tego powodu istnieje zapotrzebowanie na obiektywne, mniej inwazyjne i bardziej powtarzalne metody pomiaru. Nowe technologie powstają by sprostać temu zapotrzebowaniu.

Niniejsza praca doktorska opiera się na obserwacji gruczołów Meiboma przy pomocy NIM, a jej głównym celem jest:

- ocena wpływu starzenia na powierzchnię oka w kontekście gruczołów Meiboma;
- ocena zależności pomiędzy nowymi, obiektywnymi metodami oceny morfologii gruczołów Meiboma a innymi klinicznymi parametrami opisującymi powierzchnię oka;
- ocena i porównanie charakterystyk termalnych powierzchni oka u pacjentów z ZSO i zdrowych pacjentów przy pomocy termografii podczerwonej.

W tym celu przygotowano protokół kliniczny zawierający pełną procedurę oceny powierzchni oka, uzupełnioną o nowe metody. Protokół ten zawiera: wywiad lekarski; pomiar osmolarności łez przy pomocy osmometru TearLab Osmolarity System; automatyczne pomiary jakości filmu łzowego przy pomocy wielofunkcyjnego keratografu Keratograph 5M (K5M); pomiar temperatury powierzchni oka przy pomocy bezkontaktowej termografii podczerwonej; ocena powierzchni oka przy pomocy biomikroskopu z lampą szczelinową; pomiary barwienia rogówki i spojówki oraz czasu przerwania filmu łzowego przy użyciu fluorescein oraz barwienie powierzchni oka i tarczki powiekowej zielenią lizaminową. Dodatkowo przeprowadzono ocenę zdrowia powiek. Dla każdej powieki wykonano też NIM przy pomocy K5M. Na koniec wykonano 5-minutowy test Schirmera w znieczuleniu miejscowym i przy zamkniętych oczach.

Część eksperymentalna niniejszej pracy podzielona została na cztery niezależne badania. W pierwszym z nich zastosowano przedstawiony powyżej protokół kliniczny, zaś udział w badaniu wzięło 110 uczestników (70 kobiet i 40 mężczyzn) w wieku (średnia ± odchylenie standardowe) 44 ± 19 lat. Pomiary wykonano na jednym oku u każdego z pacjentów. Niniejszy eksperyment pokazuje, iż w populacji osób starszych występuje więcej negatywnych zmian na powierzchni oka niż w grupie osób młodych. Większość parametrów wykazała silną korelację z wiekiem. Niniejszy eksperyment pokazuje wpływ wieku na powierzchnię oka i jej związek z ZSO. Dodatkowo, grupa kobiet wykazała więcej zmian na powierzchni oka spowodowanych wiekiem niż odpowiadająca im grupa mężczyzn.

W drugim badaniu wzięło udział 161 uczestników (91 kobiet i 70 mężczyzn) w wieku (średnia ± odchylenie standardowe) 42 ± 17 lat, którzy zostali pogrupowaniu według parametru MGL, mierzonego skalą *meiboscore* zaproponowaną przez Reiko Arita et al. w 2008 roku. Dokonano porównania pomiędzy wyróżnionymi grupami oraz obserwowano korelacje klinicznych miar zdrowia powierzchni oka z MGL. Grupę o wyższym MGL cechowała wyższa średnia wiekowa, toteż w analizie uwzględniono wiek uczestników. Wyniki niniejszego badania wskazują, iż parametrowi MGL wyższemu niż 50% towarzyszy zwiększona osmolarność, zaczerwienienie oraz mierzalne uszkodzenie nabłonka powierzchni ocznej. Zauważono także, iż określając ubytki MGL i ich wpływ na powierzchnię oka, a także przy ustalaniu wartości odcięcia w diagnostyce ZSO, powinno się uwzględniać różne grupy wiekowe.

W eksperymencie trzecim użyto automatycznego algorytmu do analizy obrazów zarejestrowanych przy pomocy NIM. Algorytm ten został opracowany w Katedrze Inżynierii Biomedycznej Politechniki Wrocławskiej w celu obiektywnej oceny morfologii gruczołów Meiboma. Uwzględniono w nim kilka różnych obiektywnych miar, tj. MGL, długość i szerokość gruczołów oraz nieregularność ich kształtu. W badaniu przeanalizowano 149 obrazów przedstawiających wywinięte powieki górne uczestników poprzedniego eksperymentu. Niniejsze badanie pokazuje, iż większość parametrów zmierzona automatycznym algorytmem jest silnie skorelowana z wiekiem, zatem ich wpływ na powierzchnię oka powinien być zawsze rozpatrywany w oparciu o wiek. W niniejszych badaniach wykazano także, że nieregularność gruczołów Meiboma może być

prodromem MGL. Gruczoł Meiboma będzie dalej funkcjonował niezależnie od kształtu, a dopiero znaczny wzrost MGL będzie miał wpływ na powierzchnię oka.

W ostatnim z przeprowadzonych eksperymentów wzięło udział 86 uczestników: 48 oczu zdrowych i 38 oczu z ZSO, w średnim wieku odpowiednio (średnia ± odchylenie standardowe) 39 ± 12 lat oraz 49 ± 19 lat. W eksperymencie zmierzono temperaturę powierzchni oka przy pomocy termografii podczerwonej. Temperatura rejestrowana była przez okres 40 sekund, w trakcie których uczestnicy mogli swobodnie mrugać. Niniejsze badanie nie wykazało różnic w rozkładzie termalnym powierzchni oka na przestrzeni 40 sekund pomiędzy osobami zdrowymi i z ZSO oraz pomiędzy osobami z dwoma podtypami ZSO. Niniejsze zjawisko da się wytłumaczyć w oparciu o wpływ ciepłoty powiek na temperaturę powierzchni oka podczas mrugania. W oparciu o te wyniki można zauważyć, że pomiary temperatury powierzchni ocznej pomiędzy mrugnięciami będą miały większe znaczenie kliniczne w diagnozie ZSO.

Podsumowując, niniejsza praca doktorska przyczynia się do poszerza stanu wiedzy na temat morfologii gruczołów Meiboma i jej związku ze zdrowiem powierzchni oka. Parametry je określające są niezwykle przydatne w diagnostyce i prowadzeniu terapii ZSO. Dodatkowo, w niniejszej pracy, wykazano kliniczną przydatność termografii podczerwonej.

ABBREVIATIONS

- **AC:** Allergic Conjunctivitis
- **ADDE:** Aqueous Deficient Dry Eye
- **BR:** Bulbar Redness
- **CCD:** Charge Coupled Device
- **CL:** Contact Lens
- **CLAC:** Contact Lens Related Allergic Conjunctivitis
- **CLD:** Contact Lens Discomfort
- **DED:** Dry Eye Disease
- **DEQ:** Dry Eye Questionnaire
- **DEQ-5:** Dry Eye Questionnaire (short-version)
- **DEQS:** Dry Eye-Related Quality-of-Life Score
- **DEWS I:** First International Dry Eye Workshop (2007)
- **DEWS II:** Second International Dry Eye Workshop (2017)
- **EDE:** Evaporative Dry Eye
- **FDA:** Food and Drugs Administration
- **GCC:** Geometric Centre of the Cornea
- **IBI:** Interblink Interval
- **ICC:** Interclass Correlation Coefficient
- **ICD:** Periglandular Inflammatory Cell Density
- **IDEEL:** Impact of Dry Eye on Everyday Living
- **IOLs:** Intraocular Lenses
- **IQR:** Inter-quartile Range
- **IR:** Infrared
- **KCS:** Keratoconjunctivitis Sicca
- **K5M:** Keratograph 5M
- **LE:** Left Eye

- **LFU:** Lacrimal Functional Unit
- **LL:** Lower Eyelid
- **LLP:** Lipid Layer Patterns
- **LLT:** Lipid Layer Thickness
- **LR:** Limbal Redness
- **LWE:** Lid Wiper Epitheliopathy
- **MCLs:** Multifocal Contact Lenses
- **MG:** Meibomian Glands
- **MGAUD:** Meibomian Gland Acinar Unit Density
- **MGALD, MGALSD:** Meibomian Gland Acinar Longest and Shorter Diameter
- **MGD:** Meibomian Gland Dysfunction
- **MGL:** Meibomian Gland Loss/ Dropout
- **MOST:** Mean Ocular Surface Temperature
- **MQ:** McMonnies
- **NEI:** National Eye Institute
- **NIBUT:** Non-invasive Break-Up Time
- **NIKBUT-avg:** Average Time of All Tear Film Break-up Incidents
- **NIKBUT-first:** First Break-up of the Tear Film
- **NIM:** Non- Contact Infrared Meibography
- **Non-SS:** non-Sjögren's Syndrome
- **OCM:** Optical Coherence Microscopy
- **OCT:** Optical Coherence Tomography
- **OSDI:** Ocular Surface Disease Index
- **OST:** Ocular Surface Temperature
- **PGA:** Preserved Prostaglandin Analogues
- **QoL:** Quality of Life

- **RE:** Right Eye
- **ROI:** Region of Interest
- **SANDE:** Symptom Assessment in Dry Eye
- **SD:** Standard Deviation
- **SESoD:** Subjective Evaluation of Symptom of Dryness
- **SPEED:** Standard Patient Evaluation of Eye Dryness
- **SS:** Sjögren's Syndrome
- **TBUT:** Tear Break-up Time
- **TER:** Tear Evaporation Rate
- **TFO:** Tear Film Osmolarity
- **TFOS:** Tear Film and Ocular Surface Society
- **TMA:** Tear Meniscus Area
- **TMH:** Tear Meniscus Height
- **TMHk:** Tear Meniscus Height (Keratograph)
- **TMR:** Tear Meniscus Radius
- **UL:** Upper Eyelid
- **USD:** United State Dollars
- **WHS:** Women's Health Study

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Chapter 1

Introduction

The purpose of this introduction is to provide a wide background about several aspects of the ocular surface.

1.1 THE OCULAR SURFACE

The ocular surface is considered a well-integrated unit composed by the cornea, conjunctiva, lacrimal glands and eyelids that are interconnected [1]. It was first defined by Thoft et al.[2] in 1978 but its description was extended by Gipson [3] in 2007 in her Friendenwald lecture as: *"the ocular surface…includes the surface and glandular epithelia of the cornea, conjunctiva, lacrimal gland, accessory lacrimal glands, meibomian glands and their apical (tears) and basal (connective tissue) matrices; the eyelashes with their associated glands of Moll and Zeiss; those components of the eyelids responsible for the blink and the nasolacrimal duct".* In the same year, the *International Dry Eye WorkShop* (DEWS I) included the term of Lacrimal Functional Unit (LFU) introduced by Stern et al.[4] in 1998 as *"an integrated system comprising the lacrimal glands, ocular surface (cornea, conjunctiva and meibomian glands), lids, and the sensory and motor nerves that connect them*".

The principal function of the LFU is to preserve the integrity of the tear film, ensure the transparency of the cornea and also the quality of the image that will be projected onto the retina[4–6].The components of the LFU are directly exposed to the outside world, therefore any damage to any of these components could lead to a destabilization of the tear film resulting in an ocular surface disease termed as dry eye disease (DED).

1.2 DRY EYE DISEASE

Historically, the definition of DED has been modified and adapted several times along the years as the understanding of the condition increased. Furthermore, several difficulties were found in establish a widely-accepted definition due to a lack of understanding about the processes involved in DED.

The first formal DED definition was presented in 1995 at the National Eye Institute (NEI)/Industry Dry Eye Workshop[7] as *"Dry eye is a disorder of the tear film due to tear deficiency or excessive tear evaporation which causes damage to the interpalpebral ocular surface and is associated with symptoms of ocular discomfort".* This definition highlighted that the DED could be caused by aqueous tear deficiency but also by excessive tear film evaporation giving equal relevance to the change of quality and quantity of the tear film with respect to their normative values as a cause of DED. On the other hand, the term "disorder" was included in this definition instead of "disease" which may not reflect the true relevance of DED. In addition, the term DED is considered as synonymous with the term keratoconjunctivitis sicca (KCS). Later on, in 2006, a Delphi consensus group proposed *"dysfunctional tear syndrome"* as a new name to define DED, suggesting that this name reflects the pathophysiology events associated with this condition and also reflected the importance of both tear film quantity and quality[8]. Moreover, the experts involved in the panel concluded that treatment strategies should rely on symptoms and signs and a severity-based treatment algorithm was suggested for this purpose[8].

The following year, in 2007, the first International Dry Eye Workshop (DEWS I)[9] elaborated by the *Tear Film and Ocular Surface Society* (TFOS) proposed a DED definition centered on the clinical effects and associated signs, which was: *"a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface".* For the first time, the definition acknowledges the DED as a disease with a multifactorial nature. Discomfort symptoms were still relevant, but symptoms of transient visual disturbance were also included in this definition. On the contrary, a statement regarding the mechanism or aetiology of DED was not included.

After a decade of advances in the DED field, TFOS proposed an evidence-based definition included in the second *International Dry Eye Workshop* (DEWS II) [10]published in 2017. It was described as follow: *"Dry eye is a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles".*

This new definition still highlights the multifactorial nature of the DED but as a novelty, the loss of homeostasis of the tear film was included as a unifying element that describes the essential process in the development of DED. Regarding ocular symptoms, these remained as relevant DED feature. Furthermore, the neurosensory abnormalities (not included in last versions) have been reported to play an important role in the etiology together with the tear hyperosmolarity, tear film instability and ocular surface inflammation as reflected in the definition. Futures advances in the DED knowledge may lead to a new definition of the DED.

1.3 DED EPIDEMIOLOGY

The epidemiology data provides an essential information about the frequencies and types of diseases in a population and the factors that influence its distribution. Nowadays, the magnitude of the DED problem worldwide is better understood thanks to all the information compiled from the last thirty years of DED research. Despite this, the epidemiological studies need to face several challenges that complicate the interpretation of the epidemiological data. This could be the lack of a validated diagnostic test for DED diagnosis or even the lack of standardization in the DED definition and classification [11]. Both DEWS I & II reports published to date, which contribute a valuable information about this relevant issue.

1.3.1 DED PREVALENCE

DED is considered one of the most frequently encountered ocular morbidities seen by eye care practitioners. Additionally, it has been reported that the 25% of patients who visit ophthalmic clinics report symptoms of DED, making it a growing public health problem[12]. The prevalence in DED vary due to the definition of DED used and the characteristics of the population studied, resulting in large differences in the reported prevalence data. In 2007, according to the DEWS I Epidemiology report, the prevalence of DED ranged from 5 to 30% in individuals over the age of 50[13]. Currently, according to the Epidemiology report from the DEWS II [11], the prevalence rate varies depending on the diagnostic criteria applied. Indeed, in studies that involve symptoms (with or without signs), the prevalence ranged from 5 to 50% revealing that they are more consistent than the prevalence rates including signs (which it raises up to 75% in certain populations) [11].

According to the *Women's Health Study* (WHS) criteria, the prevalence of DED based on symptoms ranged between 14.4 and 24.4%[14–16] but the majority of the conducted studies, which applied this criterion, were performed in Asia. In addition, other studies carried out in China [17]and Japan[16] have reported that between 21 to 24% of high school students reported DED symptoms, showing higher rates than in adults. On the other hand, a study conducted in American males applying this criterion reported the lowest age adjusted prevalence of 4.3% [18]. Despite this, women showed higher prevalence than men in all studies stratified by gender.

Regarding symptomatic DED (**Figure 1**), it has been observed that it is more common in women than men, being from 1.33 to 1.74 times higher in women[19–21]. Despite this, other studies have found no significant sex difference[22,23]. Also, the symptomatic DED has been reported to be more common in Asian than Caucasian populations[11]. The highest prevalence rates of symptomatic DED have been shown on studies conducted in South East Asia, ranging from 20.0 to 52.4%[19,22,24]. The diagnostic criteria applied to these studies was based on the frequency of symptoms, having at least one of several symptoms of DED or all at the same time (such as dryness, irritation, foreign body sensation, itching, or burning). However, although applying the same diagnostic criteria, the prevalence rates reported in Spain[25], USA[26] and UK[27]

were lower (18.4%, 14.5% and 20,8%, respectively). On the other hand, studies conducted in France[28] and Iran[29] using the OSDI cut-off value above 22 as diagnostic criteria, the prevalence rates reported were very different (39.2% and 18.3%, respectively).

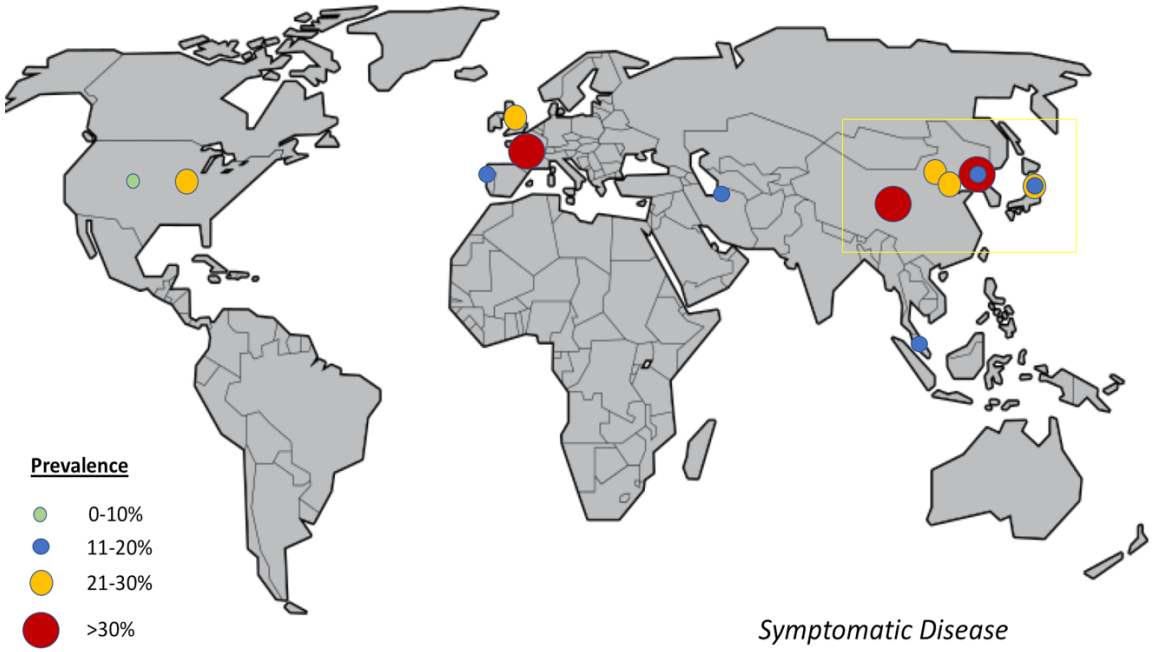


Figure 1. Prevalence map of symptomatic DED. The prevalence percentage is detailed in the legend and the red square cover the area with higher prevalence rates worldwide. **Based on "Epidemiology report" (DEWS II)**[11]**.**

When the diagnosis is only based on DED signs, the prevalence rates are variable. In addition, depending on the study, a single clinical sign or a combination of them is chosen. For example, the prevalence of DED vary between studies, from 15.6 to 85.6% for TBUT$\leq$10 seconds whereas for the Schirmer test $\leq$5 mm varies between 19.9 and 37% [22,23,25]. The differences in the prevalence rates reside in the characteristics of the population studied, poor repeatability of the tests, lack of establishing cut-off values and the different techniques used. It is important to highlight that the signs may also not always be an intrinsic feature of the DED and may reflect normal ageing or conditions other than DED. Indeed, studies previously have been found an association between ageing and an increase in positive DED signs has been found[22,23]. This increase being a factor of 10 times greater with clinical DED signs as compared with symptoms.

Currently, it is not clear how the pathological thresholds from different clinical signs could be adjusted to age. Despite this, DEWS II Diagnostic report suggest a cut-off at ± 2 standard deviations from the mean value [30].

On the other hand, when the diagnosis is performed based on a combination of symptoms and signs, the prevalence of DED ranges from 8.7 to 30.1% [25,27,28]. Nevertheless, different criteria were applied in each of the studies which makes it difficult to draw clear conclusions.

Finally, as suggested previously[13], it has been demonstrated that there is a lack of association between the prevalence of symptomatic DED and the prevalence of signs which implies that a considerable proportion of subjects are asymptomatic.

1.3.2 IMPACT OF DED ON QUALITY OF LIFE AND ECONOMY

Given the high prevalence worldwide, DED is considered to have a significant impact on the quality of life (QoL) of affected patients[31,32]. The several symptoms of discomfort and the decreased visual function associated to DED can lead to a reduction in physical functioning, loss of leisure time and deterioration of general health[33]. Indeed, DED patients present worse perception of their health and vitality in comparison to the general population, being worse when the severity is increased[34]. The QoL of DED patients is considered similar to that experienced by patients with mild psoriasis, moderate-to-severe angina or even those who undergo hospital dialysis[35–37]. Daily activities such as reading, watching television, carrying out a professional work, driving or viewing a computer screen can be limited by the blurred vision reported in DED patients. These patients are three times more likely to report difficulties in performing these activities[33]. Furthermore, it is important to mention that DED has an adverse impact on psychological health. DED patients have reported to be more anxious, depressed, psychologically stressed and showed lower scores for mental health compared to patients without DED [38,39]. Depression has been especially associated with increased severity of DED symptoms[40]. Sleep and mood seem to be affected by DED since the prevalence of sleep and mood disorders are significantly higher in patients who suffer from DED compared with those encountered with other ocular conditions as

glaucoma, retinal disease or cataracts[41]. In addition, sleep disturbance has been associated with DED being equal for men and women[42].

Apart from the impact on QoL, the direct medical care spending and the loss of productivity contribute to the DED has economic burden[43–45]. The management of DED involve the utilization of several healthcare resources such as follow-up visits with physicians, pharmalogical and non pharmalogical therapies available and surgical procedures. The global cost of the DED is difficult to address because of healthcare systems differing from country to country. For example, in the United States, the annual cost in DED management is around 3.84 billion dollarts (USD) [45] while in Singapore it is 0.15 USD millions [46]. In addition, the DED annual costs vary across Europe ranging from 0.27 million USD in France to 1.10 million USD in the United Kingdom [47]. As well as the global cost, the DED treatment cost varies by country according to the different treatment options available in each one and even in the same geographical location [48–50].

The impact of indirect costs due to absenteeism (early leaving or work absent) and presenteeism (productivity loss when employees are not fully productive during working hours) are also relevant in the economic framework. Actually, it has been reported that DED is associated to decreased productivity and days missed from work [43,44,51,52]. Future strategies should be focus on the improvement of the QoL of the DED patients leading to a reduction of the economic burden.

1.3.3 RISK FACTORS OF DED

The multifactorial nature of DED is well-recognized. For this reason, the knowledge about the risk factors provide relevant and valuable information about the pathophysiological mechanisms involved, allowing advances in diagnostic methodology and therapeutic perspectives [11]. Ageing [18,25,29,53] and female sex [14,19,27,54] have been confirmed as the most consistent risk factors for DED according to large epidemiologic studies. Asian race [14,55], meibomian gland dysfunction (MGD) [56,57], connective tissue diseases and Sjögren syndrome (SS) are the risk factors that have been proven to increase the risk of DED. In the DEWS II report, all risk factors have been classified by the level of evidence (consistent, probable and inconclusive) and into “non-

modifiable" and "modifiable" in order to help on the DED management (for more details see **Figure 3 of Epidemiology report" (DEWS II)** [11]).

1.4 DED CLASSIFICATION

As well as the DED definition, the DED classification has been adapted according to the understanding of the disease. The classification schemes are useful to guide diagnosis and as a result, improve patient care applying the appropriate treatment in each particular case [10].

The NEI/Industry Workshop classification proposed in 1995 was useful and durable for over a decade. This report presented tear-deficient and evaporative as primary categories of DED and also proposed several intrinsic and extrinsic etiological factors within each category, that could contribute to DED [7]. Despite this, this classification needed to be updated because it did not reflect different aspects regarding effects on vision, pathophysiological mechanisms and the importance of the severity assessment [9].

In 2003, the Committee from the 14th *Congress of the European Society of Ophthalmology* proposed the "triple classification"[58]. Later on, in 2005, it was updated and presented in three separate schemes based on the etiopathogenesis, the glands and tissues targeted in DED and disease severity [59]. This approach was considered attractive to DEWS committee who took many conceptual aspects derived from this scheme for the next classification scheme that was proposed later in 2007. Nevertheless, from the Delphi Panel report, the DEWS committee considered the severity grading [8]. Therefore, the classification scheme [9] presented in the DEWS I report in 2007 (**Figure 2**) was the result of the knowledge obtained from the reports presented at the 14th *Congress of the European Society of Ophthalmology* together with the Delphi panel report.

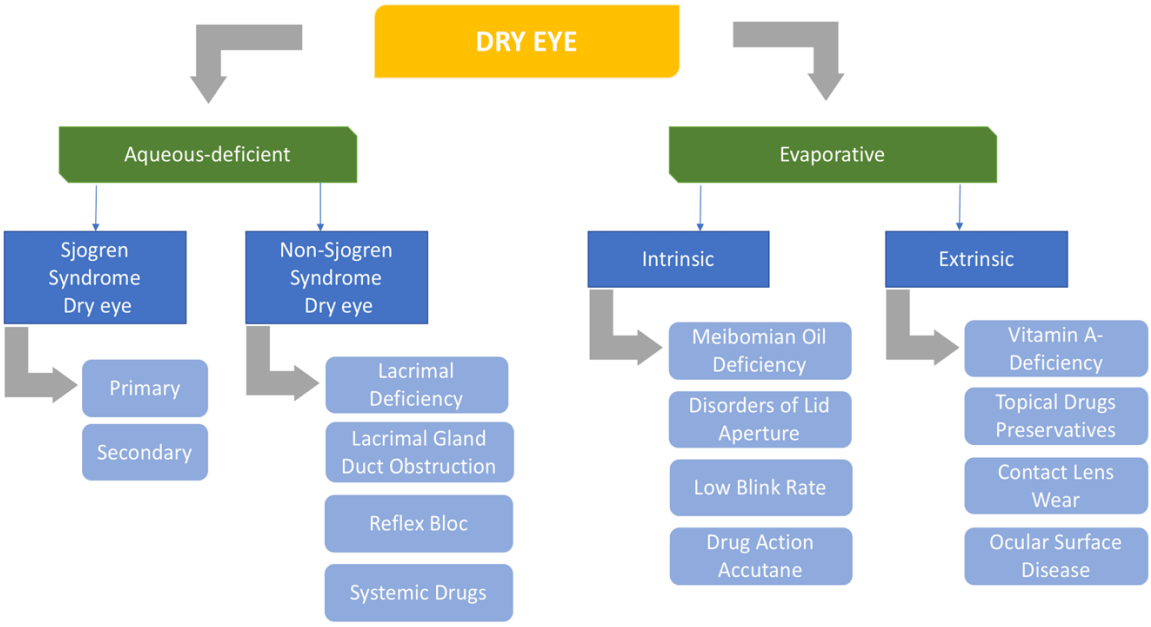


Figure 2. Dry eye classification from DEWS I report published in 2007. **Based on "Definition and Classification report" (DEWS II)**[10]**.**

The etiopathogenic classification developed and presented in the DEWS I preserved the two DED major categories as "aqueous deficient dry eye" (new term for "tear deficient") (ADDE) and "evaporative dry eye" (EDE). Suffering from any of these DED types lead to an alteration of the quality and/or the composition of the tear film, which result in a reduction of the tear stability and an increase of tear film osmolarity. Furthermore, it has been reported that these DED types are not mutually exclusive, being possible that these entities could co-exist at the same time [10].

ADDE is characterized by a failure of lacrimal tear secretion that can be caused by lacrimal acinar cells destruction or dysfunction [60,61]. As a result of it, the tear osmolarity increase leading to a stimulation of inflammatory mediators. Furthermore, ADDE is subdivided into Sjögren syndrome (SS) and non-Sjögren syndrome (non-SS). The SS is described as an exocrinopathy in which the lacrimal and salivary glands are targeted by an autoimmune process. In addition, other organs are also affected by the SS. Both lacrimal and salivary glands are infiltrated by activated T-cells, which cause acinar and ductular cell death and hyposecretion of the tears or saliva [62].

On the other hand, non-SS is a form of ADDE developed by several causes as the reduction of the reflex lacrimal secretion, deficiencies in the main and secondary lacrimal gland and obstruction of the lacrimal gland ducts [62].

EDE is characterized by an excessive water loss from the exposed ocular surface in the presence of normal lacrimal secretory function [62]. As observed in **Figure 2**, the causes that lead to EDE have been described as intrinsic (drug actions, MGD, low blink rate, etc) which affect lid structures or dynamics and extrinsic (vitamin A deficiency, topical drugs preservatives, etc) where ocular surface disease occurs due to some extrinsic exposure [62].

As well, DED can be categorized as episodic or chronic. The episodic DED occurs when environmental or visual tasks with reduced blinking overwhelm the stability of the tear and produce symptomatic DED. On the other hand, chronic DED persists continuously with symptoms and possible damage to the ocular surface [63].

The current DED classification scheme published in the DEWS II is showed in **Figure 3.**

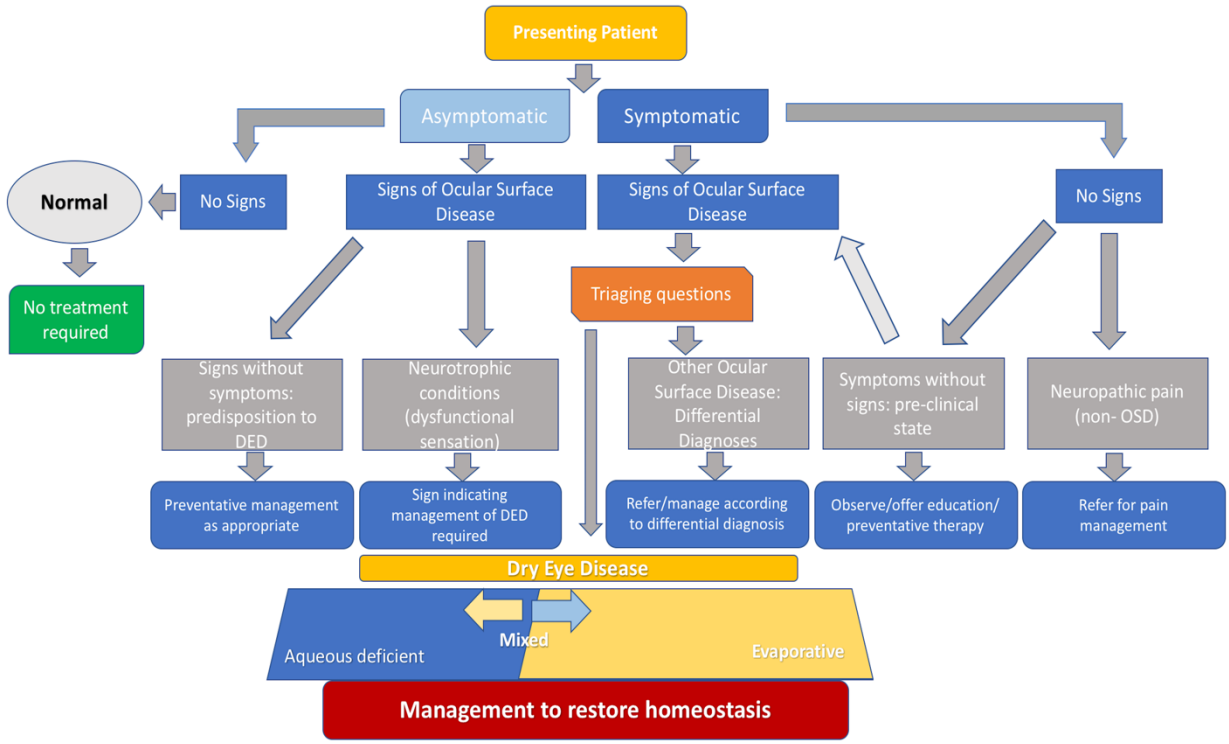


Figure 3. Current DED classification. **Based on "Definition and Classification report" (DEWS II)** [10]**.**

This new classification considers that ADDE and EDE exist on a continuum rather than as separate entities [10]. Triaging elements have been included in order to avoid misdiagnosis of DED in the presence of other ocular surface diseases. Accuracy in DED diagnosis is critical since it increases the chance of establishing a successful treatment. For this reason, a clinical decision algorithm based on the DED pathophysiology has been incorporated. In addition, this classification considers the cases where patients present marked signs without DED symptoms and those who experience DED symptoms without DED signs [64,65].

It is important to highlight that this classification is based on the predominant aetiologies ADDE and EDE. Despite that, EDE cover a large area on the diagram, according to the current understanding, an evaporative component to DED is more common than an ADDE component. Indeed, MGD has been considered the most common cause of DED in both clinic and population based studies [56,66,67].

1.5 THE PATHOLOGY OF DED AND THE VICIOUS CIRCLE

As confirmed previously in the DEWS I, the core mechanisms of DED are the tear film hyperosmolarity along with tear instability. It is well-known that tear hyperosmolarity is caused due to reduced lacrimal secretion (ADDE) or excessive evaporation of tears from the ocular surface (EDE). It has been shown that these two DED subtypes may co-exist but despite this, the progression of any form of DED may lead to additional evaporative features. The pathological process starts when the tear hyperosmolarity generates a cascade of events that lead to ocular surface damage by the release of inflammatory mediators and proteases. Tear hyperosmolarity together with the inflammatory mediators can cause goblet cell and epithelial cell loss and damage to the epithelial glycocalyx. Additionally, these mediators reinforce the ocular surface damage leading to punctate epitheliopathy of DED together with tear film instability which leads to early tear film break-up. As well, the tear break-up aggravates and magnifies tear hyperosmolarity and it completes the so-called Vicious Circle (**Figure 4**) of events that lead to ocular surface damage, perpetuating the disease [68].

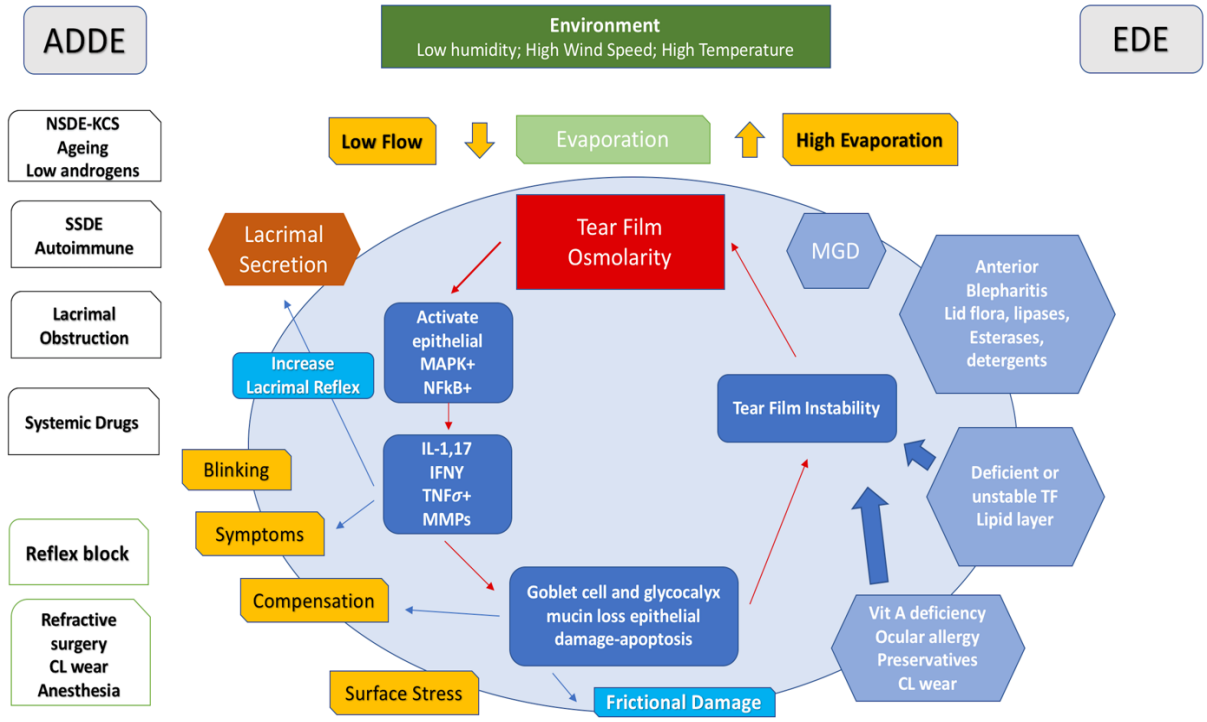


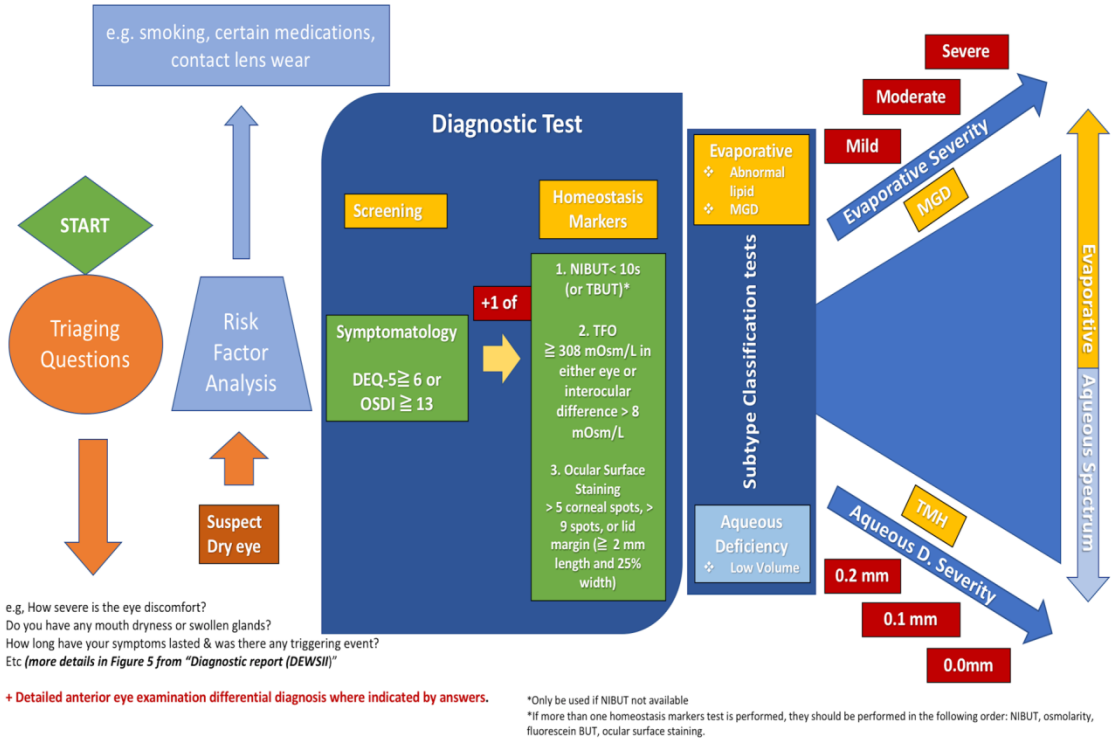
Figure 4. Representation of the vicious circle of DED. The cycle of events is shown at the centre of the figure where the core driver of DED is tear film hyperosmolarity. **Based on "Pathophysiology report" (DEWS II)** [68]**.**

1.6 DED DIAGNOSIS

An accurate clinical diagnosis is necessary in order to distinguish DED from other ocular conditions which may share several characteristics (such as signs and symptoms) with DED. In addition, according to the diagnosis, the adequate treatment is applied, therefore, performing a correct diagnosis is a key to success.

The combination of different diagnostic tests [62,69]has been used in order to assess both symptoms and signs since most of the tests show poor repeatability as well as low sensitivity and specificity.

The DED experts that participated in the Diagnostic report (DEWS II)[30] recommend performing the diagnosis evaluating at least the "*homeostatic marker*" tests after performing a screening questionnaire. On the other hand, in order to identify the DED subtype or specific aspects (such as inflammatory markers or environmental triggers), it is suggested to perform additional DED metrics.



TFO: Tear film osmolarity (mOsms/L); TMH: Tear meniscus height (mm); NIBUT: non-invasive tear film break-up time (seconds); MGD: Meibomian gland dysfunction; TBUT: Tear break-up time(seconds); OSDI: The Ocular-surface-disease-index (scores); DEQ-5: Dry Eye Questionnaire (scores)

Figure 5. DED diagnostic test battery proposed by TFOS. Based on **"Diagnostic report (DEWSII)"**[30].

As observed in the **Figure 5,** symptoms and at least one positive result of the markers of homeostasis should constitute the diagnosis of DED. Also, it is always recommended that the tests should be performed from the least to the most invasive. The clinical procedural is detailed as follows:

1.6.1 SYMPTOMS ASSESSMENT

The questionnaires are useful screening tools for DED diagnosis. Furthermore, these are relevant for monitoring the progression of the DED and response to treatments [12]. Indeed, the questionnaires allow the evaluation of different aspects of DED symptomatology, including severity, ocular surface discomfort, vision symptoms, health-related QoL or even their effect on daily activities. For this reason, it is highly recommended that at the beginning of the clinical examination the eye care practitioner administers a validated DED questionnaire. **Table 1** gives a summary of the most frequently used and validated DED questionnaires in the clinical studies and practice.

Table 1. Most used DED questionnaires in the clinical studies and practice.

Questionnaire	Year	Description	Other comments
McMonnies (MQ) [70,71]	1986	- 14 items. - DED screening and assess risk factors. - Items include age, sex, contact lens wear, previous diagnosis of DED and triggers. - It assesses the frequency of symptoms dryness, grittiness, soreness, redness, tiredness and medications used.	Clinical studies
Ocular Surface Disease Index (OSDI) [72]	2000	- 12 items (0-4) - Measures frequency of symptoms, environmental triggers and vision related quality of life.	Clinical studies
Dry Eye Questionnaire (DEQ) [73,74]	2002	- 21 items - It includes categorical scales of prevalence, frequency, diurnal severity and intrusiveness of symptoms in a typical day over a one-week recall period.	Epidemiological and clinical studies

Women's Health Study (WHS) questionnaire [37]	2003	- It consists of only 3 questions. 1) Previous diagnosis of dry eye from clinician? *(yes or no).* 2) How often eyes feel dry (not wet enough)? *(constantly, often, sometimes, or never)* 3) How often eyes feel irritated? (*constantly, often, sometimes, or never)* The person is considered positive for DED with reported rates of disease based on symptoms of dryness and irritation at least often and/or a physician's diagnosis of DED, as reported by the participant.	It has been reported to have similar sensitivity and specificity as a 16-item instrument Epidemiological studies
Standard Patient Evaluation of Eye Dryness (SPEED) [75]	2005	- 4-question survey to assess the frequency and severity of patient DED symptoms. - It monitors diurnal and longer-term symptom changes over the course of three months.	It has been shown to exhibit good validity, unidimensionality, objectivity and consistency when compared with the DEQ, MQ questionnaire, OSDI and SESoD questionnaire. Epidemiological studies and clinical practice.
Symptom Assessment in Dry Eye (SANDE) [76]	2007	- 2 questions - Short and intuitive based on a visual analog scale that quantifies both severity and frequency of DED symptoms.	Clinical practice
Subjective Evaluation of Symptom of Dryness (SESoD) [77]	2008	- 3- items - Evaluate a patient's perception of ocular discomfort related to dryness	It has been validated against the SPEED, OSDI, DEQ, and MQ Clinical practice
Dry Eye Questionnaire (DEQ-5) [78]	2010	- 5 questions related to visual disturbance, including the frequency of visual changes, how noticeable the visual disturbance is in the morning and at night, as well as how much the visual fluctuation bothers the respondent.	It is sensitive to disease severity and is one of two instruments recommended by the TFOS DEWS II diagnostic methodology report.

Impact of Dry Eye on Everyday Living (IDEEL) [79]	2011	- 57 questions evaluating DED symptom bother, impact on daily life and treatment satisfaction	Epidemiological and clinical studies
Dry Eye-Related Quality-of-Life Score (DEQS) [80]	2013	- 15 items - An overall summary scale and 2 multi-item subscales: impact on daily life and bothersome ocular symptoms	Clinical practice

Currently, the OSDI is the most widely used DED questionnaire and has been accepted by the *Food and Drugs Administration* (FDA, USA) for its use in clinical trials. This questionnaire was developed by the *Outcomes Research Group* (Allergan Inc) in 1997 and consists in 12-item which assess DED symptoms and also the effects on vision-related function in the past week of the patient's life. It has shown good specificity (0.83 %)and a moderate sensitivity (0.60%) when differentiating between patients with DED and normal subjects [72]. The questionnaire has 3 categories related to ocular symptoms, vision-related function, and environmental triggers that the patients can rate with a 0 (none of the time) to 4 (all of the time) scale. The final OSDI score range from 0 to 100 where scores 0 to 12 representing normal, 13 to 22 representing mild DED 23 to 32 representing moderate DED, and greater than 33 representing severe DED.

The SANDE is a short questionnaire which quantifies both severity (from *"very mild"* to *"very severe"*) and frequency (from *"rarely"* to *"all of the time"*) of DED symptoms through 2 questions that use 100-mm horizontal linear visual analog scale. Furthermore, the questionnaire has demonstrated to have good test reliability (Interclass Correlation Coefficient (ICC) $0.53 - 0.76$) when repeated assessments were done, suggesting that it is useful in detecting changes in symptoms over time (SANDE version II) [76].

The SPEED questionnaire has been developed to evaluate symptoms and monitor both diurnal and longer-term symptom changes over the course of 3 months [75,81]. The score of the SPEED questionnaire is derived by summing the scores from the 8 items that assess frequency and severity (from 0 to 28). Symptoms such as dryness, scratchiness, grittiness, irritation, burning, watering, soreness, and eye fatigue are included in the questionnaire. This questionnaire has shown a good sensitivity (0.90%) and specificity (0.80%) [82].

MQ questionnaire is one of the most commonly used DED questionnaires to screen and to assess DED risk factors. It is composed of 14 questions that include DED risk factors such as age, gender, previous DED treatments, DED-related symptoms (both primary and secondary to environmental triggers), and systemic conditions associated with DED (dryness of mucous membranes, arthritis, thyroid disease, and medication use). Previously, several studies have reported different values of sensitivity ($34-98\%$) and specificity ($36-97\%$) for the McMonnies questionnaire [70,83].

The DEQ-5 is the short version of the DEQ questionnaire comprising 5 questions only. It has been shown that it is sensitive to disease severity, being one of the two tools recommended by the TFOS (DEWS II). Four questions of the questionnaire are related to visual disturbance which includes the frequency of visual changes, how noticeable the visual disturbance is in the morning and at night, together with how much the visual fluctuation bothers the patient [78].

1.6.2 TEAR FILM STABILITY

The tear film instability has been included in the new revised definition of DED and its assessment is fundamental for DED diagnosis [10,84]. The measurement of the tear film stability can be performed invasively or non-invasively. Traditionally, the tear film stability has been assessed through the tear film break-up time (TBUT). This method previously described by Norn[85] consists of the instillation of unpreserved fluorescein drops in the eye and measure the time to the first break-up of the tear film after a complete blink using a slit-lamp with a cobalt blue filter. Any ocular damage should be avoided by instilling the fluorescein at the outer canthus. In addition, in order to reduce the variability of the test and obtain more reliable data, three repeated measurements are recommended. Also, TBUT should be evaluated between 1-3 minutes after the instillation in order to obtain optimal results [86]. Normal TBUT values are found between 20 and 30 seconds while the values for DED ranges from a cut-off time of less than 10 seconds and less than 5 seconds when smaller [87,88]. Despite this, other studies have found that the average in healthy middle-aged patients is lower than 10 seconds [89]. In the case of individuals with SS, the sensitivity and specificity reported were 72.2% and $61.6\ \%$, respectively [90]. One of the most important limitation of this

method is the fact that the instillation of fluorescein itself provoke tear film instability. For this reason, it is recommended to control the amount in order to obtain more repeatable results [91]. Despite the limitations of this method, TBUT remains as one of the most widely used diagnostic tests for DED in a clinical practice [92,93].

Indeed, in order to overcome the drawbacks associated with TBUT, Mengher et al.[94] described the non-invasive tear film break-up time (NIBUT). Its measurement has become more relevant in both clinical practice and research along the years. It involves the measurement of the time between a complete blink and the first deformation of the image obtained from the specular reflection of an illuminated grid pattern from the tear film [95]. Currently, several corneal topography systems allow the NIBUT measurement through observation of the placido rings reflected on the ocular surface [96]. In addition, there are also commercial software available assessing the NIBUT automatically. Indeed, the NIBUT values obtained with automated systems have found to be shorter than those obtained subjectively [97,98]. For this reason, it is important to establish a standardized methodology when conducting NIBUT measurements [99]. Normal NIBUT values have been found to range between 10 and 15 seconds, being indicative of DED a cut-off value of less than or equal to 10 seconds [100]. Depending on the technique used, the sensitivity and specificity of the NIBUT oscillate between $82-84\%$ and $76-94\%$, respectively[101,102].

1.6.3 TEAR FILM OSMOLARITY

The tear film osmolarity (TFO) has been widely studied in clinical settings since it has been considered as the single best metric to diagnose and classify DED [103]. Furthermore, TFO has shown the highest correlation to disease severity of clinical DED diagnosis tests [104]. Tear hyperosmolarity is one of the central events in the vicious circle of DED and one of its core mechanisms, leading to reduced cell volume and increased concentration of solutes [105].In the past, the systems used to measure the TFO have been limited to research laboratories and only trained technicians could obtain the samples[106]. Clifton and vapor pressure osmometers are examples of techniques usually used to measure the TFO which showed high sensitivity and specificity as well as accuracy [107]. Despite this, a new handheld instrument developed

by TearLab was introduced to perform the TFO measurement in an easy way, allowing its use on the clinical setting [108]. This device analyses around 50 nl of tear film and in a few seconds provides the TFO reading.

Mean TFO values in normal subjects range from 270 to 315 mOsm/L (average of 300 mOsm/L) [108–110]. In addition, the normal variation between both eyes is around 6.9 ± 5.9 mOsm/L [111]. On the other hand, taking into account different degrees of DED severity and aetiologies, the mean values range between 297 and 337 mOsm/L (average of 315 mOsm/L). TFO has been reported to increase with disease severity [112], therefore it is possible to be classified as normal (302.2 ± 8.3 mOsm/L), mild-to-moderate (315.0 ± 11.4 mOsm/L) and severe (336.4 ± 22.3 mOsm/L). In the case of subjects affected severely, both TFO average, variability between eyes and visits are increased [113].

TFO readings have been seen to be highly variable and it is believed it is due to compromised homeostasis of the DED tear film. For this reason, these differences between TFO readings can be considered a marker for tear film instability [110,111,113]. In the current literature about TFO several cut-off values for DED have been proposed. The sensitivity and specificity of TFO for cut-off values from 305 mOsm/L [109] to 316 mOsm/L oscillate between $64 - 91\%$ [114–116] and $78 - 96\%$ [109,117], respectively. Besides, positive predictive values using this range oscillate from 85% to 98.4% [108]. Regarding these findings, a cut-off value of 316 mOsm/L is established to better differentiate moderate to severe DED when used in parallel with other specific tests. On the other hand, the 308 mOsm/L cut-off has become a widely accepted value for use in the clinical practice to help diagnose mild to moderate subjects [110,114].

1.6.4 OCULAR SURFACE INTEGRITY

The ocular surface integrity assessment is performed using a slit-lamp biomicroscopy and vital dyes such as fluorescein and lissamine green which are extensively used in the diagnosis and management of DED. Fluorescein is the most commonly used dye since it is a hydro-soluble colorant that allows the observation of both the tear film and epithelial erosions in the conjunctiva and cornea [118]. Fluorescein should be instilled with a strip wetted with saline, but the excess saline should be shaken off to instil a

minimal volume. The optimal fluorescein staining is evaluated between 1 and 3 minutes after instillation[86], considering a positive result > 5 corneal spots [119]. The lissamine green allows the assessment of the conjunctival and lid margin damage. This dye is less toxic than the rose bengal and better tolerated [120]. In addition, under lissamine green the blood vessels and haemorrhages can be observed with greater contrast [121]. Lissamine green dye should be instilled in a similar way as fluorescein but the saline drop should be retained on the strip for at least 5 seconds to elute the dye. The optimal lissamine staining is evaluated between 1 and 4 minutes after instillation [86], considering a positive result > 9 conjunctival spots [119]. The assessment of both corneal and conjunctival staining is considered an important aspect in the clinical assessment of DED severity [104], using several grading systems such as the standardized version of the NEI/ Industry Workshop [7], Efron scales [122], Van Bijsterveld system [123], the Oxford Scheme [124], etc. It is recommended to use the same grading system during the clinical follow-up since they cannot be used interchangeably [125].

1.6.5 SUBTYPE CLASSIFICATION TESTS

These tests are performed to identify the subtype of DED according to the DED classification as well as to categorize their severity in order to help on the management of DED.

1.6.5. 1 TEAR FILM VOLUME

The tear volume is also relevant for maintaining the ocular surface balance. Therefore, its assessment is also important for DED diagnosis. Currently, the quantitative evaluation of the tear meniscus is the most direct approach to assess the tear film volume. It refers to the tear fluid accumulated within the menisci, lying at the junctions of the bulbar conjunctiva and the margins of both eyelids [126]. Tear meniscus characteristics such as height (TMH), width, radius curvature (TMR), and cross sectional area (TMA) have been widely used in clinical practice and have shown good diagnostic accuracy and correlations with other DED tests [127,128]. Traditionally, the assessment

has been performed using a slit-lamp biomicroscope equipped with a graded ocular but this technique showed numerous drawbacks. For this reason, optical coherence tomography (OCT) has been used extensively in the last ten years to measure tear volume since it is an objective and non-invasive technology. The most common tear meniscus parameters obtained with this technology are the upper and lower TMH, TMA, TMR and tear meniscus depth [97,129]. Normal TMH ranges between 0.1 and 0.3 mm and values below 0.1 mm are associated to DED [130]. Despite this, the range of both normal and DED values depends on the technique used to perform the measurement [131].

The Schirmer test allows the measurement of the tear secretion by placing a paper strip (5x35 mm) into the conjunctival sac of the temporal third of the lower eyelid. After 5 minutes, the length of the wetted part of the strip is measured [30]. The Schirmer test was described by Otto Schirmer[132] and has been widely used along the years for DED diagnosis. In addition, there are several versions of this test. The more common is the Schirmer test I performed without anesthesia which provide an estimation of stimulated reflex tear flow. Depending on the authors, the cut-off values proposed oscillate between 5 and 15 mm [117,133]. For example, a cut-off of 5mm [62] showed a sensitivity of 77% and specificity of 70% [90] while using 10 mm [134] the sensitivity and specificity were 85% and 83% [123], respectively. The Schirmer test II is performed instilling topical anesthesia and it measures the function of the basal lacrimal secretion. For this reason, it is believed that it is more objective and reliable than the Schirmer test I in DED detection[135] but there is a lack of high-level evidence data on repeatability, sensitivity and specificity [136]. The cut-off value for Schirmer test version II has been set in 5mm /5 minutes. Both version of Schirmer test should be performed with the patient's eyes closed in order to minimize variability and prevent influencing factors as vertical gaze position and eye movements [30].

1.6.5. 2 EVAPORATIVE DED COMPONENT

The evaporative component type can be assessed in the clinical setting through interferometry (by measuring the tear film thickness) and the meibography (by assessing the Meibomian gland morphology).

INTERFEROMETRY

It is possible to measure the thickness of the lipid layer of the tear film (LLT) using interferometry technique based on the interferometric patterns. The thickness of tear film ranges from 15 to 160 nm with the mean of 42 nm and the most feasible value close to 30 nm [137]. It has been suggested that the evaluation of the lipid layer of the tear film by interferometry enables monitoring of the function of meibomian glands [138]. There are many devices on the market such as the Tearscope Plus (Keeler, Windsor, United Kingdom) [139] and LipiView (TearScience, Morrisville, NC) [140] that allow the assessment of the LLT. The LipiView interferometer has been considered the first clinically available device that allow automated measurement of the LLT. Using this device, the cut-off value of 75 nm has shown a sensitivity of 65.8% and a specificity of 63.4% for detection of MGD. Despite this, its diagnostic contribution to DED has not been established yet [140]. A study conducted by Eom et al.[141] found a negative correlation between the remaining meibomian gland area assessed by meibography and the LLT measured with LipiView. On the other hand, a study conducted by Finis et al.[140] with 110 DED patients found that the number of functional Meibomian glands are significantly related to the LLT [140].

MEIBOGRAPHY

Detailed information is provided in **Section 1.7.3.**

1.7 EVAPORATIVE DRY EYE

As previously described, the EDE is characterized by an excessive water loss from the exposed ocular surface in the presence of normal lacrimal secretory function [62]. The lipid layer of the tear film **(Figure 6)** has relevant functions such as reducing the surface tension of the tear film to help the tear spreading over the ocular surface, provision of a smooth optical surface, and prevention of water evaporation [142]. Its role in the tear film stabilization is crucial because any alteration of its composition, distribution and thickness is related to DED [143,144].

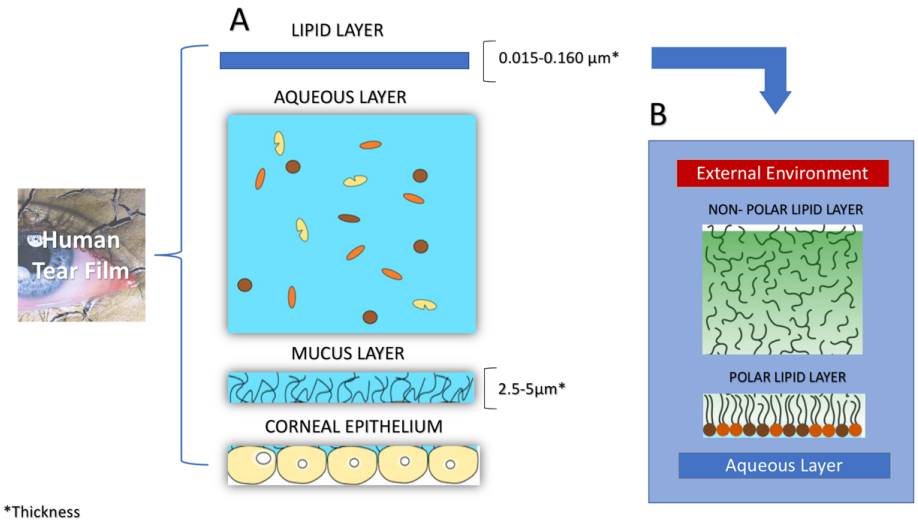


Figure 6. (A) Scheme of the tear film structure and **(B)** Scheme of the lipid layer structure. **Based on "Tear film lipid layer: A molecular level view" by Lukasz Cwiklik.**[142] **Biochim Biophys Acta. 2016.**

1.7.1 MEIBOMIAN GLANDS

Historically, these glands were named as "*meibomian glands*" in honour to the German physician and anatomist Heinrich Meibom. In 1666 he published the first detailed description and drawing of the oil glands inside the tarsus of the eyelid [145]. Despite this, it is believed that Galenus was the first who mentioned them in 200 AD [146].

Meibomian glands (MG) are modified, holocrine, sebaceous glands that are located in the tarsal plate of both upper (UL) and lower (LL) eyelids (**Figure 7**). The principal function of these glands is to secrete actively lipids and proteins that are spread over the tear film surface. This oil matter is named *meibum* and provide lubrication, promote the

stability of the tear film in order to obtain a clear optical surface and as well, to prevent its evaporation [147].

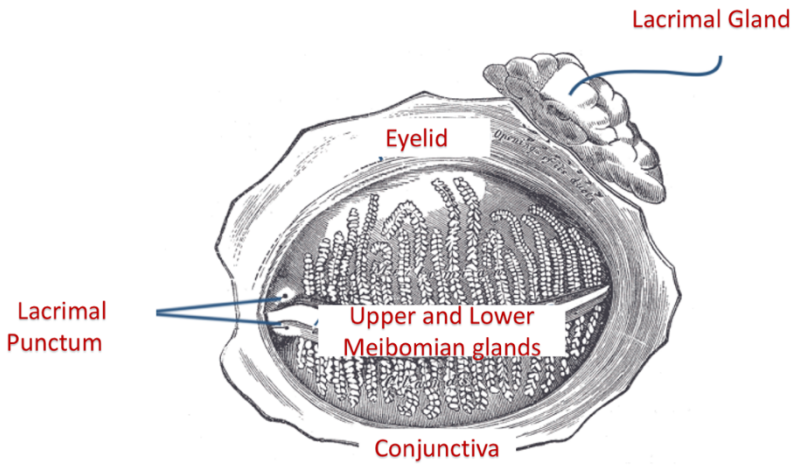


Figure 7 . Illustration representing the MG within the tarsal plates of the upper and lower eyelids.

The number of MG in the UL and LL is in median, 31 and 26, respectively. These are arranged in parallel in a single row throughout the length of the tarsal plates [148]. It has been reported that the MG from the UL are longer than the MG from LL but the latter are wider. Furthermore, the MG from the UL produces higher volume of secretion (around 26 μl) than their UL counterparts [149].

The structure of a single Meibomian gland includes clusters of secretory acini (which contain secretory cells termed as *meibocytes*), lateral ductules, a central duct, and a terminal excretory duct that opens at the posterior lid margin (**Figure 8A**).

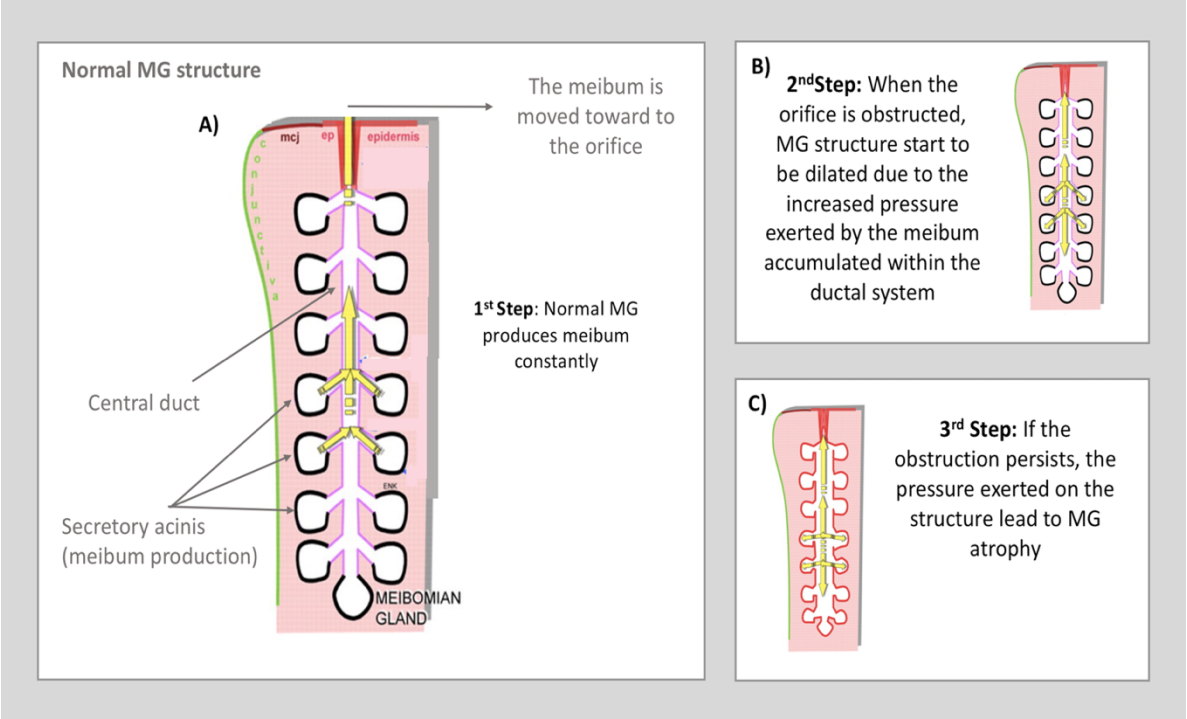


Figure 8. Progressive structural alterations of the MG in obstructive MGD. **(A)** Normal MG produces meibum constantly within the secretory acini which is transferred to the central duct and, with the help of the pretarsal orbicularis muscle and the marginal muscle of Riolan, comes out and is spread over the ocular surface. **(B)** The meibum delivery is reduced due to the obstruction and MG structure start to be dilated due to the increased pressure exerted by the meibum accumulated within the ductal system. **(C)** After a while, the pressure starts to affect the rest parts of the MG structure and the ductal epithelium turns cornified in the last stages. **Based on The International Workshop on Meibomian Gland Dysfunction: Report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland** [145]**.**

The meibum is synthesized within the secretory acini which is constantly producing oily meibum. This production generates a continuous force that allows the transportation of the meibum pushing it into the ductal system and the posterior delivery of the oily meibum onto the lid margin and the tear film [145]. In addition, the action of the pretarsal orbicularis muscle and the marginal muscle of Riolan during a blink contribute to the meibum delivery [145].

The function of the MG is regulated by androgens, estrogens, progestins, retinoic acid, growth factors and possibly by neurotransmitters since they are richly innervated with sensory, sympathetic and parasympathetic nerves [145,150,151].

1.7.2 MEIBOMIAN GLAND DYSFUNCTION

The Meibomian gland dysfunction (MGD) is considered the leading cause of EDE which is the most common subtype of DED. This term was first used by Korb and Henriquez [152] in the early 1980s as it was considered suitable for describing the functional abnormalities of the MG. In the scientific literature MGD and posterior blepharitis are used as synonymous [153,154] but these terms are not interchangeable.

The definition of this condition was established when a committee of experts from TFOS launched in 2011 the *International Workshop on Meibomian Gland Dysfunction* which collected all the available knowledge about MGD. The MGD was defined as *"a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion. It may result in alteration of the tear film, symptoms of eye irritation, clinically apparent inflammation, and ocular surface disease"*

The prevalence rate of MGD based on clinical signs in populations over the age of 40 ranges from 38 to 68% as reported by several population-based studies [19,20,155]. Furthermore, the prevalence has been seen to be higher in Asians [57] compared with Caucasians [155]. The impact of sex and age on MGD is not yet well-known [11].

Historically, more than five different MGD classifications have been proposed [153,156,157]. Currently, the MGD is classified into two major categories based MG secretion [158]: low and high delivery state, respectively. As observed in the **Figure 9**, the *high delivery state* is characterized by the release of a large amount of MG secretion in response to a pressure exerted on the tarsus. On the other hand, the *low delivery state* is sub classified into hyposecretory and obstructive. The first one is characterized by reduced MG secretion without obstruction while the second show reduced secretion due to an obstruction of the gland, being the most common form of MGD [152,159]. On the other hand, MGD may be primary when it appears without known disease association or secondary when it has associated with another disease, for example, the rosacea or psoriasis.

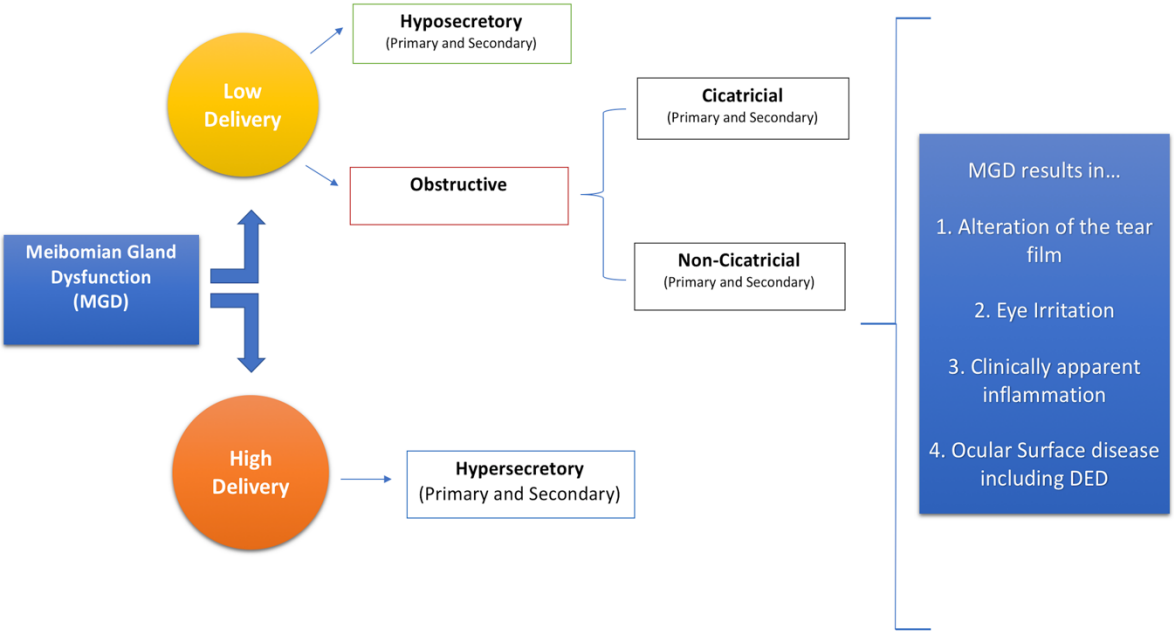


Figure 9. Classification system proposed by The International Workshop on MGD. **Based on The International Workshop on Meibomian Gland Dysfunction: Report of the definition and classification subcommittee** [158]**.**

It is suggested that the pathology starts with an obstruction of the orifice of the gland due to a hyperkeratinisation of the epithelium or increased viscosity of the meibum leading to a reduced or completely inhibited secretion. During the obstruction, the meibum is accumulated within the ductal system due to the constant production carried out by the secretory acini. The amount of meibum within the gland exerts a pressure leading to a widening of the ductal system. If this situation persists for a long time, the secretory acini will suffer the consequences of the pressure and the acini undergo atrophic changes which are visible as MG loss (or dropout) through infrared meibography [145] (**Figure 8A,8B and 8C**).

MGD is considered a symptomatic condition whose symptoms result from lid involvement and ocular surface inflammation in the absence of increased evaporation [160].

Nevertheless, the degree and extent of obstruction developed with the progression of disease result in a reduced delivery of meibum to the tear film [161] that lead to a deficiency in the lipid layer of the tear film [162], which makes lose its barrier function [144]. Changes in the lipid composition, reduced spread time and instability of the lipid

layer also contribute to the increased evaporation that finally leads to EDE [163,164]. Additionally, an asymptomatic form of MGD termed as *non-obvious MGD* has been described [165]. It is characterized by normal lid margin appearances at the slit-lamp but, in this case, due to the absence of signs the diagnosis must be based on the assessment of the quality of the expressed secretions [68].

Diagnosing MGD, it can be problematic. There is a problem in distinguishing which symptoms are related to MGD and which are related to DED since there are no specific symptoms related to this disease. Indeed, MGD is part of the vicious circle of DED [147,166] and therefore, it is not known whether MGD may lead to DED through a process of events that involves inflammation or on the contrary, the inflammation of the eyelid margin in DED may affect MG morphology and function [167]. Actually, MGD diagnosis is mainly based on slit-lamp assessment of the lid margin and ocular surface integrity, TBUT, MG expressibility and secretion quality[166]. Despite this, there is no unanimity concerning the tests that are needing to be performed for MGD diagnosis and monitoring of the treatment response. Most of the proposed tests are invasive and imply modification of the natural conditions of the ocular surface. On the other hand, they are subjective which lead to the introduction of significant observer bias because of a low degree of standardization [168]. In order to overcome these drawbacks, automated and non-invasive tests have been developed to help in MGD and DED diagnosis as NIBUT, TFO, LLT measured by interferometry and non-contact infrared meibography.

1.7.3 ASSESSMENT OF THE MEIBOMIAN GLAND STRUCTURE

1.7.3.1 IMAGING TECHNIQUES: MEIBOGRAPHY

The meibography has become the most widely used tool for both researchers and clinicians for the assessment of the MG structure. It comprises of photographic documentation of the MG under different illuminations techniques[169]. The meibography technique has evolved over the last decades in order to improve MGD and DED diagnosis providing more information about MG morphology by detecting abnormalities such as Meibomian gland loss (MGL) or dropout, shortening, dilation and distortion [170].

The first attempt of MG visualization was performed by transillumination of the everted lid. This technique was described by Tapie in 1977 [171] and it involved the use of an illumination probe (normally used during vitreous surgery) with a red light filter to transilluminate the lid and to observe it under the slit-lamp microscope (**Figure 10A**). At that time, it was the only way to observe the silhouette of the MG and obtain information about their morphology, but this technique showed several drawbacks. On one hand, the probe tip provokes heat discomfort and pain to the patient and on the other hand, the small observation area makes it difficult to capture the images of the lid.

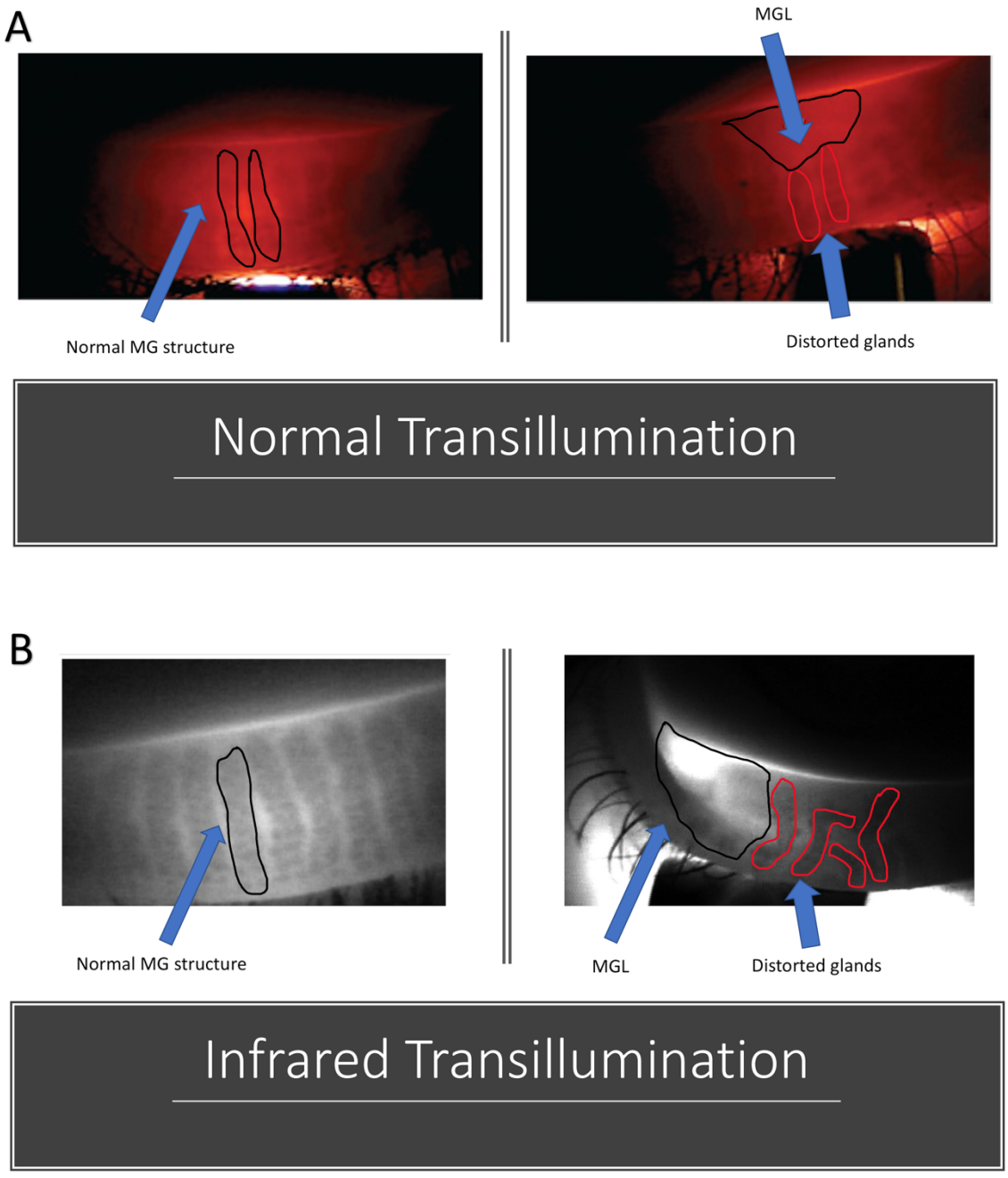


Figure 10. Different Meibography techniques **(A)** Transillumination performed in a lower eyelid with white light observed with red filter (normal vs DED). **(B)** Infrared transillumination in a lower eyelid (normal vs DED). **Based on (A) Eyelid Transillumination. Townsend WD. Cont Lens Spect. 2007 Jan;22(1):23. (B)** ***University of Iowa Health Care*** **(Ophthalmology and Vision Science).**

Later on, Tapie's technique was improved by Jester et al.[172] adapting the biomicroscopic and photographic techniques. This study was carried out in rabbits and the morphologic changes in the MG were registered by using transillumination with a

Zeiss photo-slit lamp microscope that incorporated a high-speed IR KODAK film (HIE 135-20).[172] After that, also Robin et al.[173] also used this improved technique to evaluate MGD in human which reinforced its usefulness for the MG observation.

Since IR film was expensive, the developing was time-consuming, and the results were only available after the measurement was made. Also, it was not possible to control the quality of the image [169,174], another alternative method was proposed to perform meibography. It was in 1994 when Mathers et al.[175] improved this technique developing a video-meibography system that allowed observing of the MG structure (by transillumination) in real time. Visual records of the MG from the patients were recorded using a super VHS recorder and individual frames were extracted for analysis [175]. The quality of the video images obtained was higher in comparison to IR films. Despite the advantages obtained with this technique, it is still required multiple image acquisitions in order to obtain the entire length of the lid.

In 2005, Nichols et al.[176] used digital video meibography imaging for the evaluation of the MG. They evaluated the lower eyelid using a Dolan-Jenner transilluminator and a fiber-optic guide using near-IR light and the images from the central lid were recorded using a Hitachi KP-M2R near-IR 1 chip CCD camera. This study mentioned the use of the IR light source for the first time. Later on, Yokoi et al.[177] described the use of IR light. They developed a new IR probe for meibography to enhance participant comfort and provide optimal transillumination (**Figure 10B**). The images of this study were registered by an IR CCD camera and digital video recorder. Besides, an imaging software was used to combine the MG images and with this, reduced also the number of photos also reduced that were needed for the observation of the entire eyelid.

A relevant improvement in meibography was introduced by Arita et al.[178] in 2008. They developed the non-contact infrared meibography (NIM) which consisted in a slit-lamp microscope equipped with an IR CCD video camera and an IR transmitting filter. This technique provides one image that covers the entire area of the MG from both eyelids and increase patient comfort, making it more suitable for the use in the clinical setting. With this system, the MG are detected as areas of high reflectivity and lost portions as areas of low reflectivity (**Figure 11**).

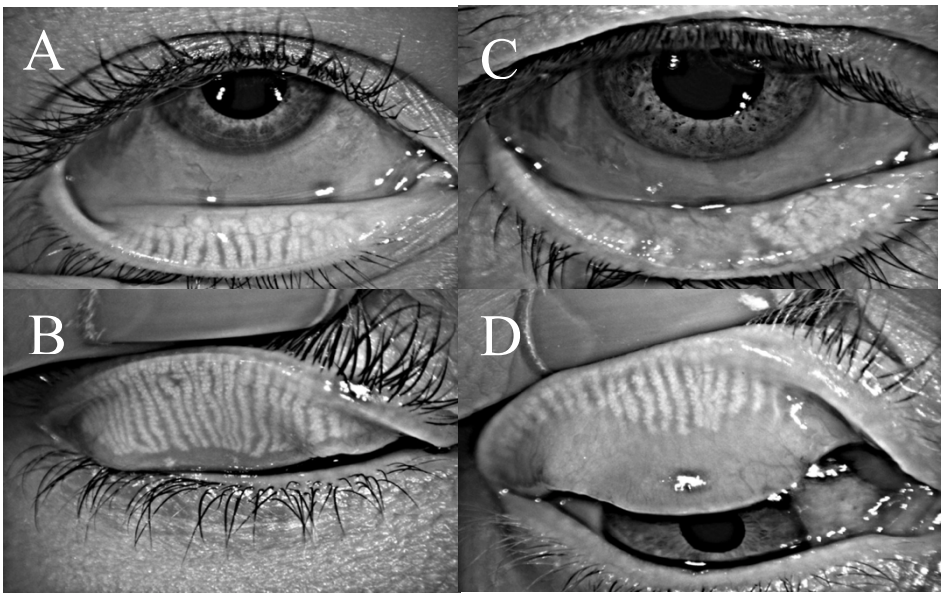


Figure 11. Meibography images from the upper and lower eyelid performed with Keratograph 5M **(A and B)** Normal subject with normal MG morphology **(C and D)** DED subject who show shortening of the MG and areas of MGL.

Few years later, Pult et al.[179] developed a novel NIM system named *Portable Non-Contact Meibography* which was based on modified IR security camera (pixel: PAL: 628(H) 582(V), 1⁄4 CCD Sensor, 802CHA CCD; Shenzhen LYD Technology Co. Ltd, Shenzhan, China). In addition, the system included a near focus adaptation by mounting a +20 diopter lens in front of the camera, allowing the visualization of the MG without using a slit-lamp. Nowadays, there are several commercially available instruments on the market that incorporate this technology for the assessment of the MG in the clinical setting.

1.7.3.2 MEIBOMIAN GLAND MORPHOLOGY

As aforementioned, NIM allows the detection of MG abnormalities such as MGL, shortening, dilation and distortion/ tortuosity of the gland. Indeed, many studies have confirmed the use of NIM for the diagnosis and evaluation of MGD [176,180,181]. The MGL or *dropout* refers to the partial or total loss of acinar tissue and it is one of the most common MG features reported in the literature [166]. Currently, there is no gold standard in the classification of MG assessed by NIM. Several scoring systems have been developed for quantification of MG features (**Table 2**). Nevertheless, there is no agreement on the number of increments that should be included in a grading scale. Regarding this, Bailey et al.[182] have suggested that a scale of fine clinical sensitivity should not exceed one-third of the standard deviation of the discrepancy indicating the use of scales with smaller increments to increase the ability to detect clinical changes. Traditionally, the quantification of the assessment has been done manually but in the last few years, semi-automatic and automatic approaches have emerged in order to obtain more reliable data.

Table 2. Grading systems developed for MG morphology assessment by meibography technique.

Author (year)	**MG feature**	**Scoring system**	**Method**
Mathers and Billborough [183] **(1992)**	MGL	Number of whole or partial MG missing from the central two thirds of the lower lid	Manual, counting
Shimazaki et al. [160] **(1995)**	MGL	Grade 0: No loss of MG Grade 1: Lost area 50% or less than the observed area Grade 2: Lost area more than 50% of the observed area *lower eyelid	Manual, counting
Pflugfelder et al.[184] **(1998)**	MGL	Grade 0: No gland dropout Grade 1: 33% gland dropout Grade 2: 34% - 66% gland dropout Grade 3: More than 66% gland dropout *lower eyelid	Manual, counting
De Paiva et al.[185] **(2003)**	MGL	Grade 0: No gland dropout Grade 1: ≤ 25% dropout Grade 2: ≤ 50% dropout Grade 3: ≤ 75% dropout Grade 4: ≤ 100% dropout	Manual, counting

McCann et al.[186] **(2003)**	MGL	Total number of absent MGs, half MG were counted as 0,5	Manual, counting
McCulley et al.[187] **(2003)**	MGL	Only the seven central MG were examined. Each gland is given a score from 0 to 4, where 0= no dropout and 4= complete dropout of that single gland. The score from each gland is summed up as a total out of 28. *lower eyelid	Manual, counting
Nichols et al.[176] **(2005)**	MGL	Number of whole MG, no credit was given for partial MG of the lower lid Grade 1: No partial glands Grade 2: Less than 25%of the image contains partial glands Grade 3: Between 25% and 75% of the image contains partial glands Grade 4: More than 75% of the image contains partial gland <u>Within-reader reliability:</u> ICC (0.92) <u>Between-reader reliability:</u> ICC (0.75)	Manual, counting
Arita et al.[178] **(2008)**	MGL (meiboscore)	Grade 0: No loss of MG Grade 1: Lost area was less than 33% of the total MG area Grade 2: Lost area was between 33% and 67% of the total MG area Grade 3: Lost area was over 67% of the total MG area Meiboscore for the upper and lower eyelids were summed to obtain a score from 0 through 6 for each eye. Interobserver mean difference: 0.08 (±0.55) on day 1 and 0.13 (±0.50) on day 2. Concordance correlation coefficient: 0.79 and 0.81 on days 1 and 2, respectively. Intraobserver mean difference: 0.04 (±0.54) and Concordance correlation coefficient: 0.79 for observer. Intraobserver mean difference: -0.09 (±0.60) and concordance correlation coefficient: 0.74 for observer 2.	Manual, counting
Pult and Riede- Pult. [188] **(2012)**	MGL	Grade 0: 0% (no partial glands) Grade 1: ≤ 25% partial meibomian glands Grade 2: between 26% and 50% partial meibomian lands Grade 3: between 51% and 75% partial meibomian glands Grade 4: > 75% partial meibomian glands <u>Intra-observer agreement</u>: 1ºobserver: κ statistics = 0.80, $p < 0.001$; 2º observer: κ = 0.40, p = 0.009; 3º observer: κ = 0.81, $p < 0.001$. <u>Inter-observer agreement</u>: 1ºobserver-2º observer: ±1.49; 1º observer-3º observer: ±0.91 and 2ºobserver-3º observer: ±1.20	Manual and semi-automatic (ImageJ)

Pult et al.[190] **(2012)**	MGL Distortion Width	Total MGL, most bent MG from both eyelid and MG width of most predominant MG.	Semi-automatic (ImageJ)
Ban et al. [191] **(2012)**	MGL	Mean value for length of the 5 central glands and proportion of MG coverage of a central area *upper eyelid	Semi-automatic (ImageJ)
Koh et al.[192] **(2012)**	Width Length	Imaging processing used for MG width and length detection MG features accurately differentiate between healthy (specificity 96.1%) and unhealthy (sensitivity 97.9%) meibography images. *upper eyelid	Semi-automatic, software developed
Arita et al. [193] **(2014)**	MGL	The ratio of the total MG area relative to the total analysis area for the upper and lower eyelid. The intra-examiner coefficients of variation for the objective analysis of upper/lower meibomian gland area in normal controls and in patients with MGD were0.59±0.26%/0.40±0.20% and 0.47±0.45%/0.44±0.31%, respectively.	Automatic, software developed
Ngo et al.[189] **(2014)**	MGL	Grade 0: 0% dropout Grade 0.5: 1%-16% dropout Grade 1: 17-33% dropout Grade 2: 51%-67% dropout Grade 3: 68%-84% dropout Grade 4: 85%-100% dropout Interobserver mean difference: 0.05 (±0.45), concordance correlation coefficient: 0.89 on day 1, and interobserver mean difference: 0.01 (±0.41), concordance correlation coefficient: 0.91 on day 2.	Manual and semi-automatic (ImageJ)
Koproswki et al. [194,195] **(2016)**	MGL	The percentage area occupied by MG is analysed in relation to the whole surface of the eyelid. The algorithm described shows a sensitivity of 99.3% and specificity of 97.5% in the diagnosis of meibomian glands and is insensitive to parameter changes.	Fully automatic, software developed

MG: Meibomian glands, MGL: Meibomian gland loss or dropout

**preferred eyelid in the study*

1.7.3.2.1 CHANGES TO MEIBOMINA GLANDS IN VARIOUS CONDITIONS REVEALED BY NIM

1.7.3.2.1.1 AGE

It has been well-documented that MGL increases with age in normal subjects [178,191,196] and it is not necessarily in response to the presence of obstructive MGD [197]. Indeed, significant positive correlation between age and the MGL was detected, indicating that the number of MG declines with age [178]. These findings remark that ageing is a relevant risk factor for the development of MGD.

1.7.3.2.1.2 CONTACT LENS

The MG morphology has been assessed in contact lens (CL) wearers. Several studies have shown that CL wear negatively affects the condition of the glands [198–200] (**Figure 12**) whereas other studies have found no relation [201,202]. A study conducted by Arita et al.[199] found higher meiboscore in CL wearers compared to control subjects and as well, the duration of the CL wear was significantly correlated with the meiboscore. A multivariate analysis found that the meiboscore was correlated with age in the CL wearers [201]. Another study conducted by Alghamdi et al.[203] found that the morphology and function of the MG were related to the duration of the CL wear. As well, they observed that the MG did not deteriorate after 2 years of CL wear and despite their discontinuation, the MG morphology did not improve.

Tang et al.[204] after assessing 60 eyes from 60 CL wearers and 21 control eyes found that CL wear longer than 3 years was associated with MGL. The possible hypothesis behind the MGL observed in CL wearers could be the chronic friction provoked by the CL [199]. In addition, MG distortion was also observed in 57 of 58 subjects who used overnight orthokeratology and also developed papillary hypertrophy [205]. Further studies are needed regarding overnight CL wear and its impact on MG morphology.

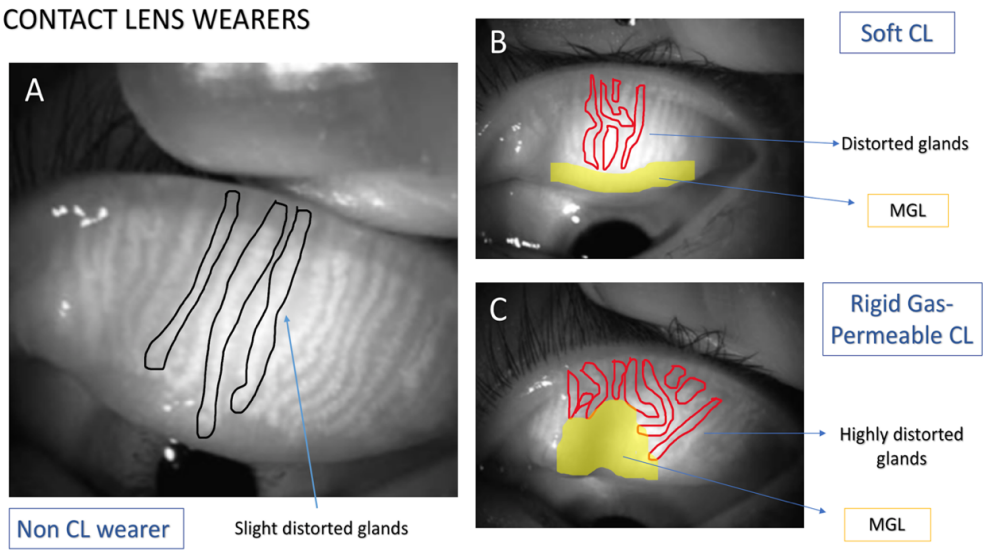


Figure 12. Representative meibography images obtained from **(A)** A non-CL wearer with total meiboscore of 0 **(B)** Soft CL wearer with total meiboscore of 1 and **(C)** Rigid gas-permeable CL wearer with total meiboscore of 4. **Based on "Meibomian Gland Dysfunction and Contact Lens Discomfort". Arita et al.**[206]**. Eye Contact Lens. 2017**

1.7.3.2.1.3 PATHOLOGIES

Both meibometry and NIM have revealed morphological changes in glaucoma patients, leading to DED [207]. MGL and low secretion expressibility are some of the most common features in glaucoma patients that use preserved anti-glaucoma drugs [208]. Arita et al.[209] found that the meiboscore was significantly higher in the eye which was under glaucoma treatment. On the other hand, a study conducted by Agnifili et al.[210] assessed the MG features in medically controlled glaucoma treated with preserved or preservative-free drugs. Their study showed a significant reduction of the mean acinar density and area, a greater secretion reflectivity, and a higher interstice inhomogeneity in patients who used preserved prostaglandin analogues (PGA). Furthermore, a recent clinical study found the association between PGA and MGD [211]. The prevalence of MGD found in this study was higher in patients treated with PGA (92.0%) compared with those receiving non-PGA therapy (58.3%). Besides, patients treated with PGA showed the obstructive MGD (95.7%) [211].

MG distortion (**Figure 13**) has been found in higher frequency in patients with perennial allergic conjunctivitis (AC) (45%) than in control subjects (8%) [212]. In

addition, the MG function of those patients have shown to be impaired. It is believed that inflammatory changes in the conjunctival tissue might induce pressure on the MG in the tarsus [145,213].

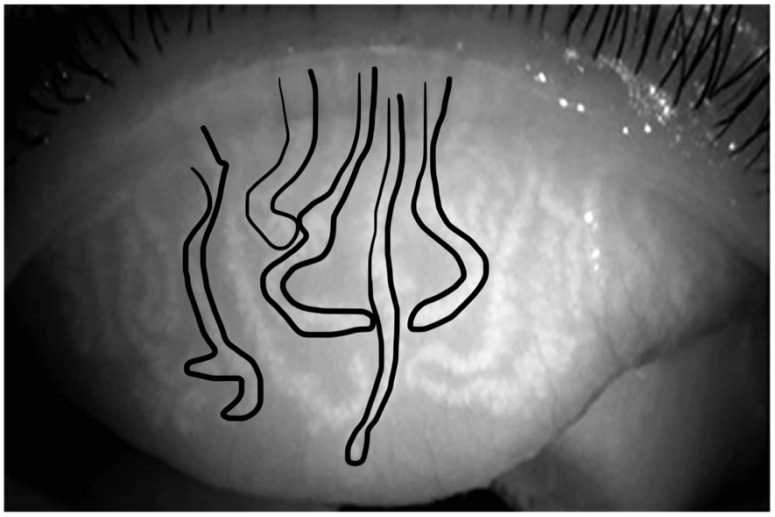


Figure 13. Distortion of the MG from the UL obtained from a patient with allergic conjunctivitis by NIM. **Based on "Functional Morphology of the Lipid Layer of the Tear Film". Arita et al.** [138]**. Cornea. 2017**

Additionally, changes have been observed in the MG morphology of patients with other pathologies such as chalazion [214,215] rosacea [216,217], ocular graft-versus-host disease [218], phlyctenular keratitis [219], granular corneal dystrophy type 2[220], vitiligio [221]and diabetes mellitus (type 2) [222]. Moreover, it has been reported that the radiotherapy [223] and chemotherapy [224] treatments affect the MG morphology.

1.7.3.2.2 DIAGNOSTIC VALUE OF MEIBOMIAN GLAND LOSS

Previously, Arita et al.[180] found that the MGL was significantly higher in obstructive MGD patients compared to controls. In addition, they showed that the symptoms, lid margin abnormalities and the meiboscore were valuable for distinguishing MGD patients and normal patients (with a sensitivity of 84.9% and specificity of 96.7% for MGD diagnosis). Another study found that the level of MGL was also able to differentiate MGD subjects from subjects with ADDE [225]. Previous studies found significant correlations between MGL and some tear film parameters (such as TBUT [191,226,227], non-invasive tear film break-up time (NIBUT)[190], lipid layer thickness (LLT) [141], Schirmer test [228], MG expressibility [226], corneal staining [227]) and

subjective symptomatology (OSDI [179]and McMonnies[229]), suggesting its possible diagnostic value [167]. However, others studies concluded that assessing the MGL alone as clinical parameter has not enough DED diagnostic value, and it should be interpreted jointly with other clinical parameters [66,180,226]. Currently, the diagnostic value of meibography in DED need further study [30]. Other MG morphology features are being studied in order to obtain more information to improve DED and MGD diagnosis.

1.7.3.2.3 MEIBOGRAPHY LIMITATIONS

Despite all advantages that this technology can provide, it is important to point out some limitations. The short wavelength IR used by the commercial IR meibography systems do not allow the penetration of the palpebral conjunctiva and in some cases, the assessment of MG could be difficult. For example, MGD patients normally show thicker palpebral compared to healthy patients [230], therefore it may affect the MG evaluation. In addition, the images do not have in depth information because they are two-dimensional. Also, different contrasts in the image obtained with different devices could introduce errors in the evaluation of the MGL [189].

1.7.3.2.4 OTHER TECHNOLOGIES FOR MG MORPHOLOGY ASSESSMENT.

In the last few years, several methods have been used for the observation of the MG structure despite the fact that they not being designed for MG assessment particularly. For example, the confocal laser microscopy allows the in vivo assessment of the histopathology of many ocular surface conditions [231,232] and recently has been useful as a supplementary diagnostic tool. The confocal laser is an invasive method and requires the instillation of topical anaesthetic. This method was first applied to the palpebral conjunctiva to observe the MG structure in 2005 [231,233] and it demonstrated that it is able to provide images of the MG acini. MG parameters such as MG acinar unit density (MGAUD), MG acinar longest and shortest diameter (MGALD, MGALSD) and periglandular inflammatory cell density (ICD) have been extracted from the observation through confocal laser microscopy. These parameters have been well correlated with the MGD severity, demonstrating to be useful for MGD assessment

[234]. The sensitivity and specificity values of these parameters for the cut- off values established were 90% and 81% for MGALD, 86% and 96% for MGASD, 100% and 100% for ICD, 81% and 81% for MGAUD [89]. Furthermore, this technology has provided information about morphological changes in patients with SS [232], effect of the ageing [235], differences in MG morphology in CL wearers [198] or even the effectiveness of MGD treatment [236].

Another method, the OCT has been used to obtain 3D and volumetric images of the MG structure [237] **(Figure 14).** This technology allows the visualizing deeper regions of the MG due to its longer-wavelength light which improves imaging depth [238]. The OCT provides images of the acini, ducts and MG lesions that are observed in the deeper layer but the observation is limited, requiring a long time to obtain the entire area of the MG [230,238]. The OCT recently has been used for histologic examination, also named as optical coherence microscopy (OCM) [239], which provides high-resolution transverse imaging and large field-of-view. This technology may provide more insight about the pathophysiology based on structural changes.

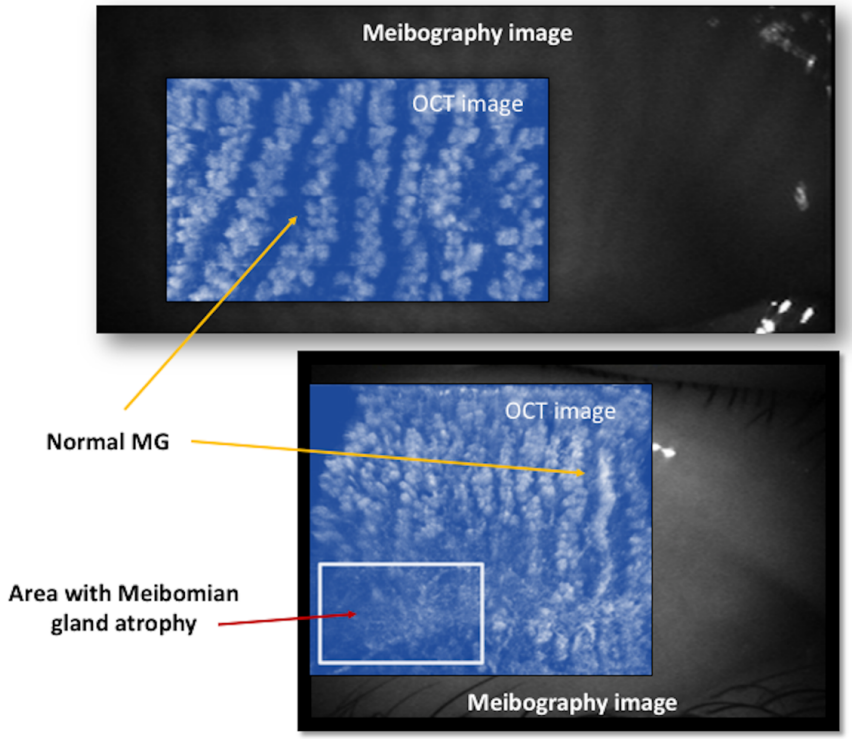


Figure 14. Images of the MG obtained with NIM and OCT **(A)** Normal MG **(B)** Increased atrophy of the MG (*white*). **Based on "Morphological evaluation for diagnosis of dry eye related to meibomian gland dysfunction" Yoo YS et al.**[238]**. Exp Eye Res. 2017**

Nevertheless, these methods mentioned above present some limitations. One of these limitations, in the case of the confocal laser microscopy, is its high cost that prevents introducing it in clinical practice. In addition, it has a small field of view, thus the image obtained is from a very small area. Also, this technology requires prolonged assessments in order to achieve the images and it would require placing the patients in very uncomfortable situations (since it is requires the instillation of topical anaesthetic and remain with the eyelid everted and the ocular surface exposed for a long period of time) [240]. Regarding OCT, the scan depth is limited to approximately 1–2 mm due to scattering and absorption of light by biological tissue. OCM method is usually performed in a research context since it allows the cellular imaging in human tissues without the use of extrinsic contrast agents or obtain images from a novel materials [241].

1.8 NEW TECHNIQUE FOR OCULAR SURFACE ASSESSMENT

Currently, the DED diagnosis and monitoring are still challenging tasks for clinicians[242] due to the multifactorial nature of DED. Most of the clinical tests used for its diagnosis show some limitations such as: semi-invasive, subjective, show insufficient sensitivity and specificity and low repeatability [62]. Additionally, there is a lack of agreement between clinical signs and severity of DED symptoms making the DED diagnosis more difficult [243,244]. Thus, more objective, less invasive, repeatable methods and technologies have been developed in order to obtain more valuable information for DED diagnosis.

1.8.1 THE ROLE OF THE TEMPERTURE IN CLINICAL DIAGNOSIS

Traditionally, the temperature has been used for clinical diagnosis since it has proved to be a good indicator of general health [245,246]. The human body is capable of maintaining the constant temperature through a physiological process called thermoregulation[247]. This regulation is vital for the normal performance in the human body, therefore changes of temperature by a few degrees could be considered as a clear sign of a possible disorder[248]. Historically, the temperature as a scientific indicator of illness was established in 1868 where the range between 36.3 and 37.5°C was considered as normal temperature and temperatures beyond this were considered as an indication of illness[249].

The measurement of the temperature has evolved through time with the advancement of the technology and discoveries related to the light. One step forward was the discovery of infrared radiation in 1800 which opened new horizons in the field of temperature measurement[245]. The diagnostic importance of temperature measurement by infrared (IR) thermography was mentioned in 1934 [250] and it was the start point to the application of this technique in the medical field. The several advantages of the IR thermography such as speed, real-time imaging, no harmful effects, non-contact and non-invasive which makes it an attractive diagnostic tool[251]. Indeed, this technique has been widely used in the medical environment for breast cancer

detection [252], dentistry[253], dermatology[254], diagnosis of rheumatologic diseases[255] and vascular disorder[256], among others.

1.8.2 INFRARED THERMOGRAPHY FOR TEAR FILM DYNAMIC ASSESSMENT

As previously mentioned, the IR thermography has been proposed as a diagnostic tool to detect pathological and physiological changes in the eye [257] allowing the study of the ocular surface temperature (OST) [258] increasing accuracy, resolution, and speed[259,260]. The OST changes registered using IR thermography reveal the nature of the tear film and its stability [261,262]. As reported by TFOS, the assessment of the tear film stability helps to identify the loss of homeostasis that is crucial in the development of DED [30]. Therefore, this technology allows the DED assessment [263,264]. Different approaches have been proposed for DED evaluation such as the measurement of the temperature of the geometric center of the cornea (GCC)[259,261], the mean ocular surface temperature (MOST) [264], relative differences in temperature across the ocular surface[263], nasal and temporal conjunctiva temperature[259], temporal and nasal limbus temperature[265] and maximum and minimum temperature of the region of the studied area [265]. The evidence in the current literature shows that the cooling rate of the ocular surface is faster in subjects with DED than in normal eyes, which is assumed to be a result of a greater rate of tear film evaporation (**Figure 15**).

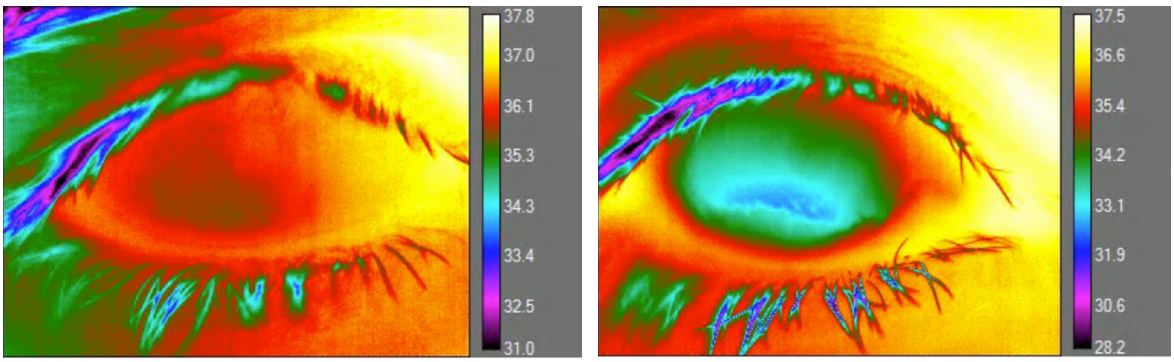


Figure 15. Examples of thermal images of **(Left)** An eye showing normal temperature of the ocular surface **(Right)** An eye showing a cooling of the ocular surface due to a higher evaporation of the tear film.

The quality and the stability of the tear film can be evaluated by tear evaporimetry. The rate of water loss of the aqueous phase or the evaporation rate (TER) from the exposed ocular surface has been widely studied in tear dynamics [266]. Several research studies have found increased TER in subjects with DED (both ADDE and EDE) [267,268] and MGD [234,269] while others did not find differences [270]. As well, blinking is considered an essential function of the eye because is responsible for spreading tears, mucin, and lipids over the ocular surface, maintaining the eye's moisture and its protection from the external environment. The interblink interval (IBI) has been demonstrated that together with other parameters related to the blink, to be useful for distinguishing between healthy subjects and patients with DED [88,271,272]. Currently, there is an emerging interest in the analysis of the OST through IR thermography as a non-invasive technology due to its high potential for DED diagnosis.

1.9 JUSTIFICATION, HYPOTHESES AND OBJECTIVES

This research work belongs to the working package of the EDEN project entitled *"Objective non-invasive diagnostic tools".* The general aim of this working package is to develop new metrics to objectively and precisely assess and analyse the LFU.

1.9.1 JUSTIFICATION

For completeness, some aspects related to the motivation behind the doctoral work is reiterated. As previously explained, DED is a multifactorial disease of the ocular surface considered one of the most frequently encountered ocular conditions seen by eye care practitioners. Nowadays, DED is estimated to affect between $5 - 50\%$ of the worldwide population [11]. Furthermore, the prevalence of DED increases linearly with age which makes DED a growing public health concern as the global population of older people is expected to be more than double its current amount by 2050. According to DED classification, ADDE and EDE are the two major DED types and are considered to exist on a continuum rather than as separate entities [10]. Despite this, according to the current DED understanding, an evaporative component is more common than an ADDE component. Currently, the MGD is considered the leading cause of EDE. This condition has been may result in alteration of the tear film, symptoms of eye irritation, clinically apparent inflammation and ocular surface disease. Therefore, any change that occurs in the morphology of the MG or in the secretion of these glands has an important clinical impact. Currently, the NIM has become the most widely used tool for both researchers and clinicians for the assessment of the MG structure. Many studies have confirmed the use of this technology for diagnosis and management of MGD [176,180,181]. Indeed, NIM allows the detection of MG abnormalities such as MGL, shortening, dilation and distortion of the glands. The MGL or *dropout* refers to the partial or total loss of acinar tissue and it is one of the most common MG features reported in the literature [166] Currently, there is no gold standard in the classification of MG assessed by NIM but most of the classification are based on the MGL. Previous studies found significant correlations between MGL and some tear film parameters (such as TBUT [191,226,227], NIBUT [190], LLT [141], Schirmer test [228], MG expressibility [226], corneal staining

[227]), subjective symptomatology (OSDI [179] and McMonnies [229]), suggesting its possible diagnostic value [167]. However, other studies concluded that assessing the MGL alone as clinical parameter has not enough DED diagnostic value, and it should be interpreted together with other clinical parameters [66,180,226].

Additionally, the multifactorial nature of the DED makes it difficult for the eye care practitioners to carry out a correct diagnosis and monitoring [242]. For this reason, more objective, less invasive, and more repeatable methods and technologies have been developed in order to obtain valuable information for DED diagnosis.

1.9.2 HYPOTHESES

The hypotheses of this research work are:

- Information revealed by NIM can be used for assessing changes in the morphology of the MG (such as loss of glandular tissue, shortening and distortion of the glands) related to DED.
- Changes in MG structure can affect the ocular surface integrity.

1.9.3 OBJECTIVES

The following objectives were established:

Objective 1 (Main)

To study the relationship between the MGL revealed by NIM and the ocular surface parameters.

Objective 2 (Secondary)

To assess the effect of ageing on the ocular surface parameters and to study its role on the DED diagnosis.

Objective 3 (Secondary)

To study the relationship between new objective MG morphology parameters and the ocular surface parameters.

Objective 4 (Secondary)

To study and compare the thermal characteristics of DED and healthy subjects using non-contact IR thermography.

1.9.3.1 EXPECTED RESULTS

The expected outcomes are as follow:

- Understanding the impact of ageing in the ocular surface and to know the ocular surface characteristics of different age groups.
- Understanding the role of the MG in the DED.
- Verifying the classification based on MGL.
- To know the impact of other MG morphology parameters apart from MGL on the ocular surface.
- Gaining knowledge about the tear film stability through ocular surface temperature assessment using a non-contact infrared thermography camera.

Chapter 2

Material and Methods

The present chapter describes the clinical protocol, methodology, material and the statistical analysis conducted for each study included in this doctoral thesis.

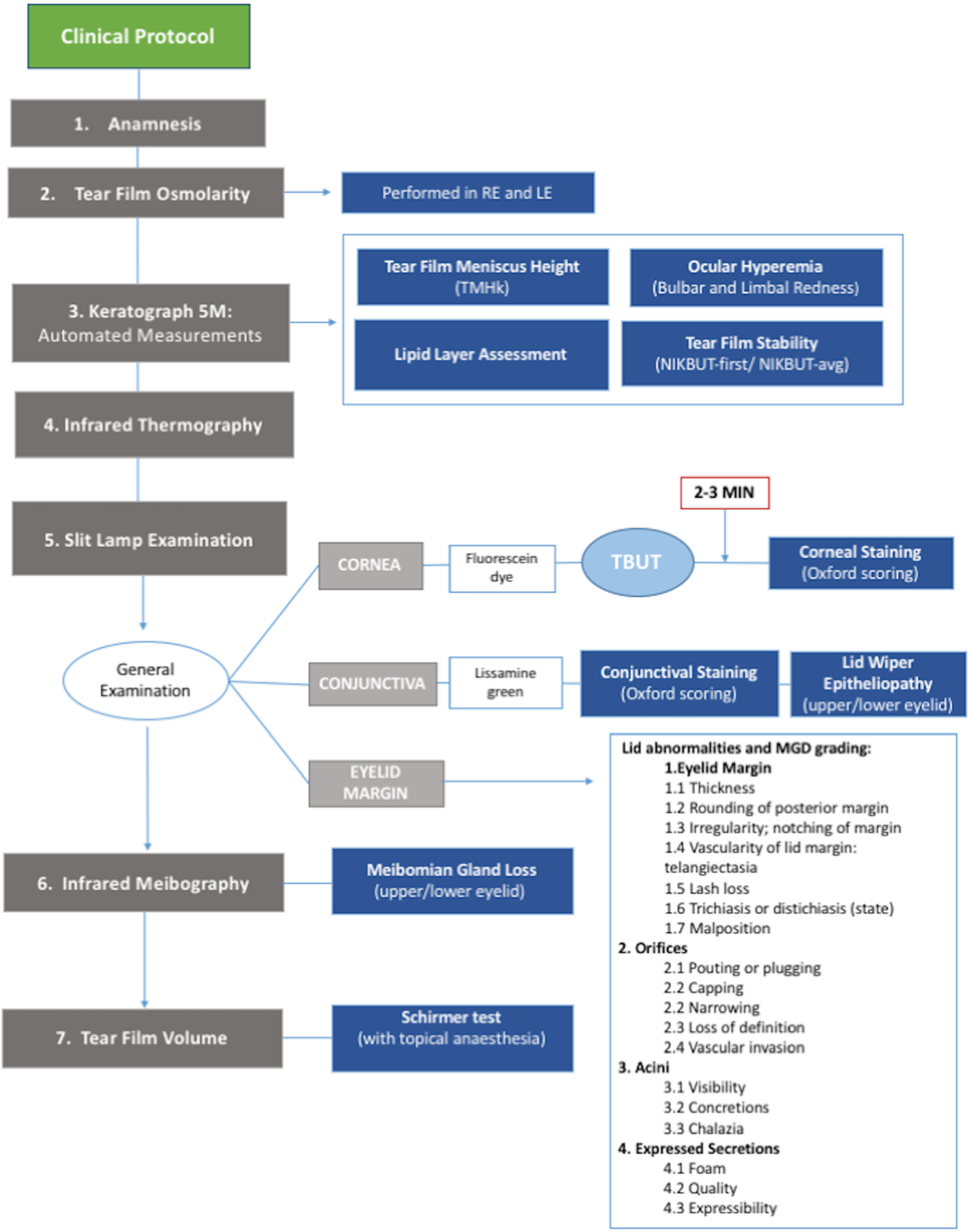
The present prospective clinical study was reviewed and approved by the Ethics Committee of San Carlos University Hospital (Madrid) and adhered to the tenets of the Declaration of Helsinki (see **Annex 1**). Written informed consent was obtained from all included participants after explanation of the purpose and consequences of the study (see **Annex 2**).

The study was carried out in the Optometry Clinic of the Faculty of Optics and Optometry at Complutense University of Madrid (UCM, Madrid, Spain). Participants were recruited via email notices sent to the institutional e-mails from the UCM academic community and advertisements placed on noticeboards in the Faculty of Optics and Optometry.

2.1 CLINICAL PROTOCOL

The research work undertaken in this doctoral thesis required designing a clinical protocol which is described in detail in **Figure 16**. Only the right eye (RE) of each participant was assessed. One eye was chosen in order to avoid a possible bias since DED affect equally both eyes and both are assessed under the same conditions. The design of this clinical protocol was elaborated with the objective of performing a comprehensive assessment of the health state and integrity of each component of the ocular surface. In order to obtain reliable information, all the clinical measurements were performed from the least to the most invasive in order to minimize the effect of the previous measurements. Therefore, the clinical examination starts with a medical interview, allowing the researcher to have a start point about the general ocular state of the participant. The tear film osmolarity is performed following the medical interview because, this way, it is possible to obtain more reliable readings before performing the Keratograph 5M measurements. Despite non-invasive measurements by Keratograph 5M, tear film test involves maintaining the eye open for almost 24 seconds. This usually leads to an excessive tear production of the eye in order to compensate for the dryness during the recording. Therefore, the excessive water content may change the osmolarity of the tear film, obtaining false results. After Keratograph 5M measurements, are performed the thermal registration of the ocular surface is measured. Before that, the participant rests for 5-10 minutes in order to allow tear film to recover from the last measurements and obtain more reliable data. This resting time is used for the participants to fill in some of the DED questionnaires. In addition, the thermal recording is recommended to be performed after 20 minutes [273]when the participant is acclimated to the room and to the surrounding environmental conditions. Once the thermal registration is performed, the biomicroscopic general assessment is carried out with diffuse illumination to carefully observe the features of the eyelids manually by touching the eye. At this point, the invasive measurements are performed. After the overall ocular surface is assessed and fluorescein dye is applied in order to evaluate the tear film stability as well as the corneal staining. The staining is assessed two minutes after the application of the dye, in order to observe the tear film when the fluorescein is on its higher peak of brightness and also, when the participant's eye has filtered or

absorbed some quantity of dye through their lacrimal system. Excessive fluorescein dye and water in the eye could lead to less reliable readings. Following the application of the dye, lissamine green is applied to assess the conjunctival integrity and the eyelid margin. After this, meibography is performed of both eyelids using K5M. The action of the eyelid eversion is normally uncomfortable for the participant, therefore it is performed carefully and also in the minimal time. Due to the invasiveness of the last test, the participant rest again for 10-15 minutes in order to allow tear film system to recover. As previously mentioned, this resting time is used for the participants to fill in the DED questionnaires. Besides, the application of the DED questionnaires at different times during the examination visit ensure the reliability of the information obtained by the questionnaires since they usually show similar results related to DED. Finally, a drop of topical anaesthesia is instilled and the Schirmer test is performed for 5 minutes with the eyes closed. At the end of the visit, if the participant has a DED symptoms caused by the measurements, artificial eye drops is provided to relieve their symptoms.



RE: Right eye; LE: Left eye; NIKBUT-fr: First break-up of the tear film; NIKBUT-avg: Average time of all tear film breakup incidents; TBUT: Tear break-up time; MGD: Meibomian Gland Dysfunction

Figure 16. Flow diagram of study protocol.

2.1.1 Participant Selection

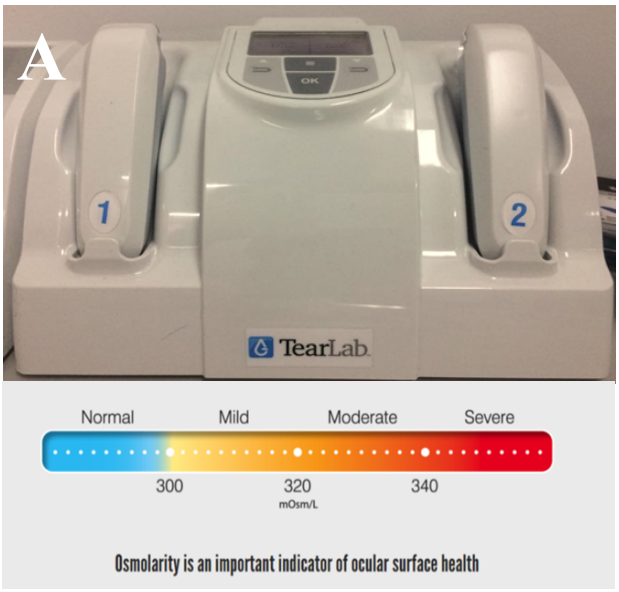
Participants were required to be 18 years of age or older, be able to complete the questionnaires, understand the procedures and obtain an evaluable meibography image of the UL and LL. They were excluded if they had a history of any active ocular disease(s) different from DED and MGD (e.g., corneal ulcers, herpes simplex keratitis, etc), uncontrolled severe systemic disease that may have affected the eye (e.g.,SS, diabetes type II, dermatological diseases, etc), ocular surgery or trauma that could affect the tear distribution, eyelid margin abnormality and/or use of contact lens wear within the week before performing the clinical examinations.

2.1.2 Medical Interview

Before starting the study, a detailed medical interview was carried out in order to obtain the relevant information of each participant. Information about age, gender, general health state (systemic history), ocular history, ocular family history, current and past medication or treatments and additional information were registered in the clinical evaluation sheet (see **Annex 3**).

2.1.3 Tear Film Osmolarity

TFO was measured using the TearLab Osmolarity System (TearLab Corp, San Diego, CA, USA) in both eyes of each participant according to the manufacturer's instructions. It was conducted as a first test in order to avoid reflex tearing or the instillation of any dye that could affect the results. The device was calibrated before each measurement using the check cards provided by the manufacturer. The participant was seated with the chin tilted upward and eyes directed towards the ceiling. In order to proceed with the tear collection, the device pen was positioned just above the lower eyelid avoiding contact with the globe during the measurement (**Figure 17**). Afterwards, the device is returned to the electronic base to obtain the osmolarity reading. One measurement per eye was performed (based on Tomlinson et al.[274]) but only the RE and the difference between the eyes (inter-eye variability) of each patient were included in the analysis.



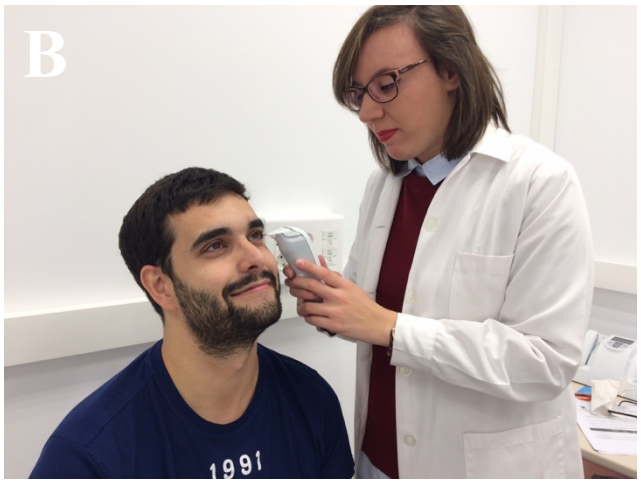


Figure 17. A) TearLab device and and TFO scale. **Image courtesy of Edouard Laffosse.**
B) The researcher performing the TFO test.

2.1.4 Keratograph 5M Automated Measurements

Automated measurements were performed using the Keratograph 5M (K5M; Oculus GmbH, Wetzlar, Germany) equipped with a modified tear film scanning function. Three measurements of the tear meniscus height (TMHk), first break-up of the tear film (NIKBUT-first), the average time of all tear film breakup incidents (NIKBUT-avg), bulbar redness (BR) and limbal redness (LR) were obtained automatically by K5M software according to the manufacturer's instructions (**Figure 18**). All the K5M measurements were performed under dimly lit room conditions.

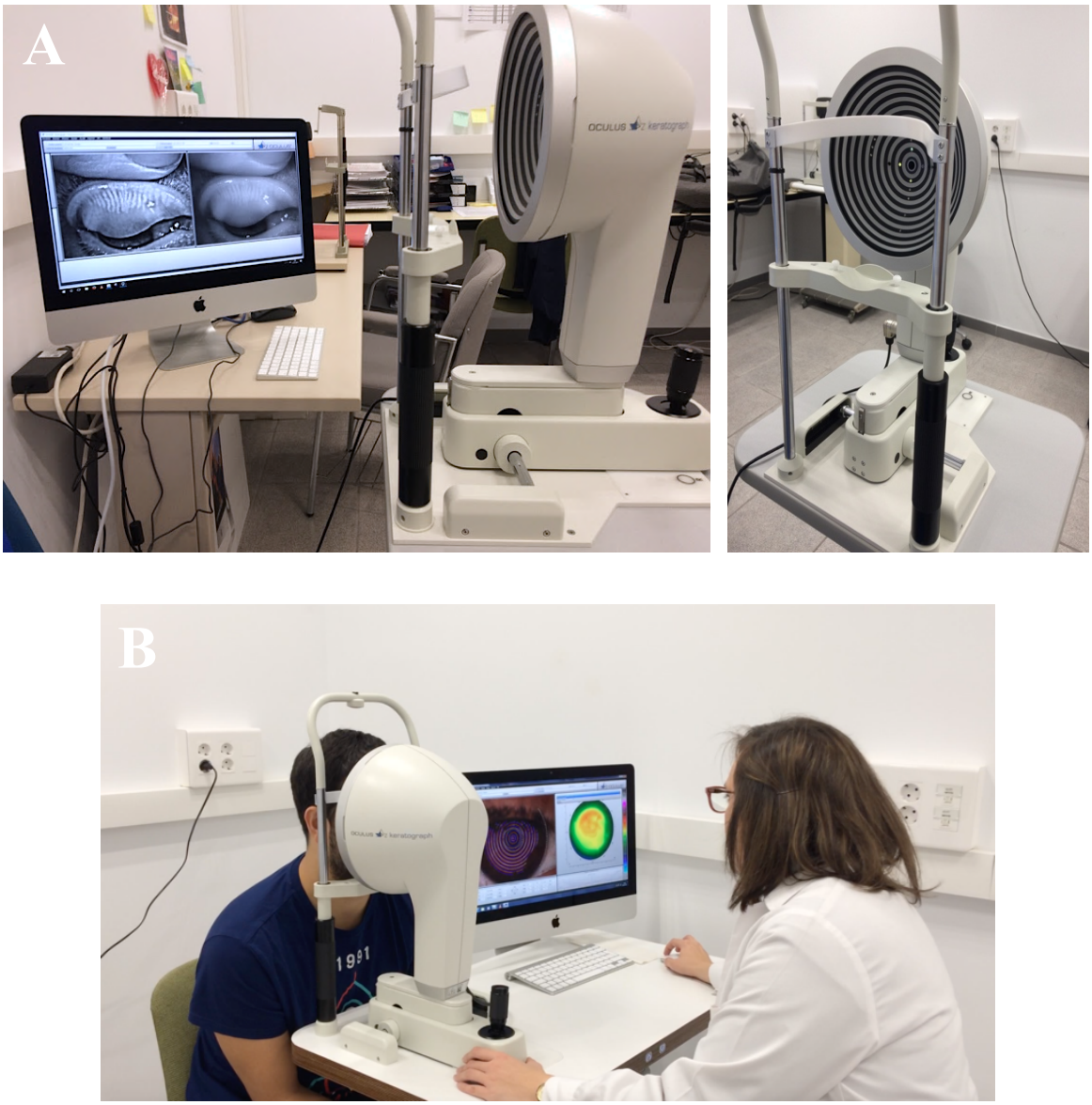


Figure 18. A) Keratograph 5M device and components **B)** Researcher performing K5M tests.

2.1.4.1 Tear Film Meniscus Height

During tear meniscus height measurement with K5M (TMHk), participants were instructed to blink naturally while looking at the target in the device. Once the images were registered by the researcher, the inferior TMHk was measured perpendicularly to the lid margin at the central point relative to the pupil center using an integrated ruler. Three reading were obtained and averaged for analysis (**Figure 19**).

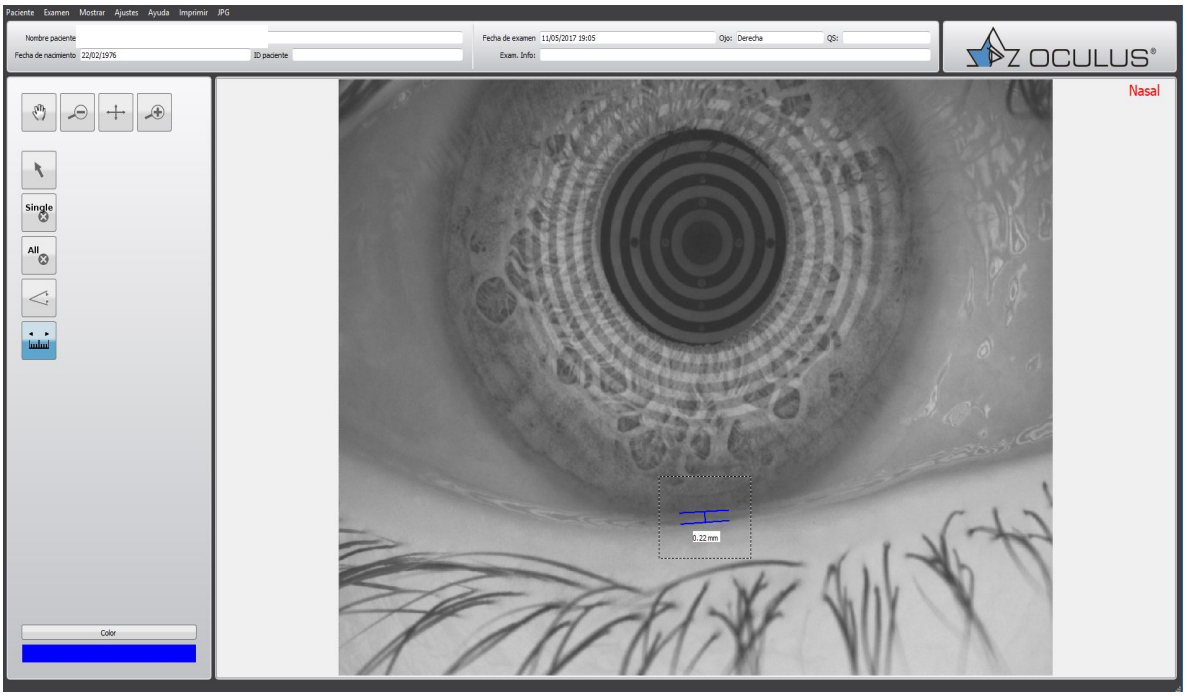


Figure 19. Representative output of TMHk performed with the K5M (*blue callipers*).

2.1.4.2 Ocular Redness

During ocular redness measurement, participants were instructed to look at the light target maintaining the eye open for few seconds **(Figure 20A).** The K5M system scanned automatically the image of the exposed conjunctiva automatically and generated a score based on the area percentage ratio between blood vessels and the bulbar conjunctiva (precise to 0.1 unit). The degree of redness, nasal and temporal, is based on the JENVIS grading scale (**Figure 20B**). Three images were obtained and the redness scores from the nasal, temporal and total conjunctiva were averaged for the analysis.

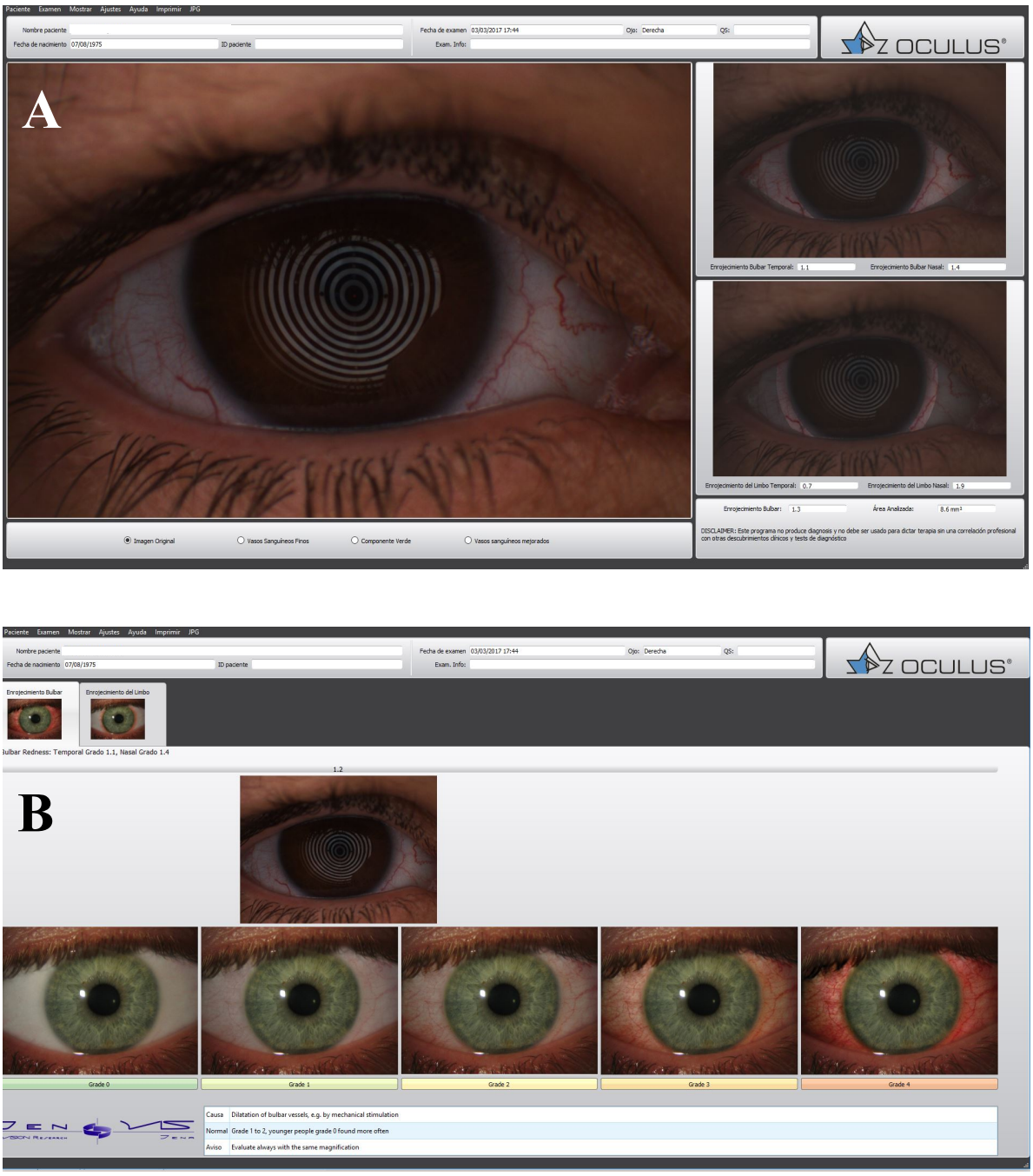


Figure 20. A) Representative output of the ocular redness performed with the K5M **B)** JENVIS grading scale.

2.1.4.3 Lipid Layer Assessment

During lipid layer assessment, participants were instructed to look at the light target and blink naturally while a digital video was recorded. The lipid layer interferometric patterns (LLP) were observed and assessed in real time between multiple blinks (**Figure 21**). The lipid layer of each participant was classified subjectively based upon its appearance as described by Guillon [275] (0= None or not visible; 1= Meshwork (open/tight); 2 = Wave (Meshwork and Wave); 3 = Amorphous (Wave and Amorphous) and 4 = Colours (Wave and Colours/ Amorphous and Colours and Colours) (**Figure 22**).

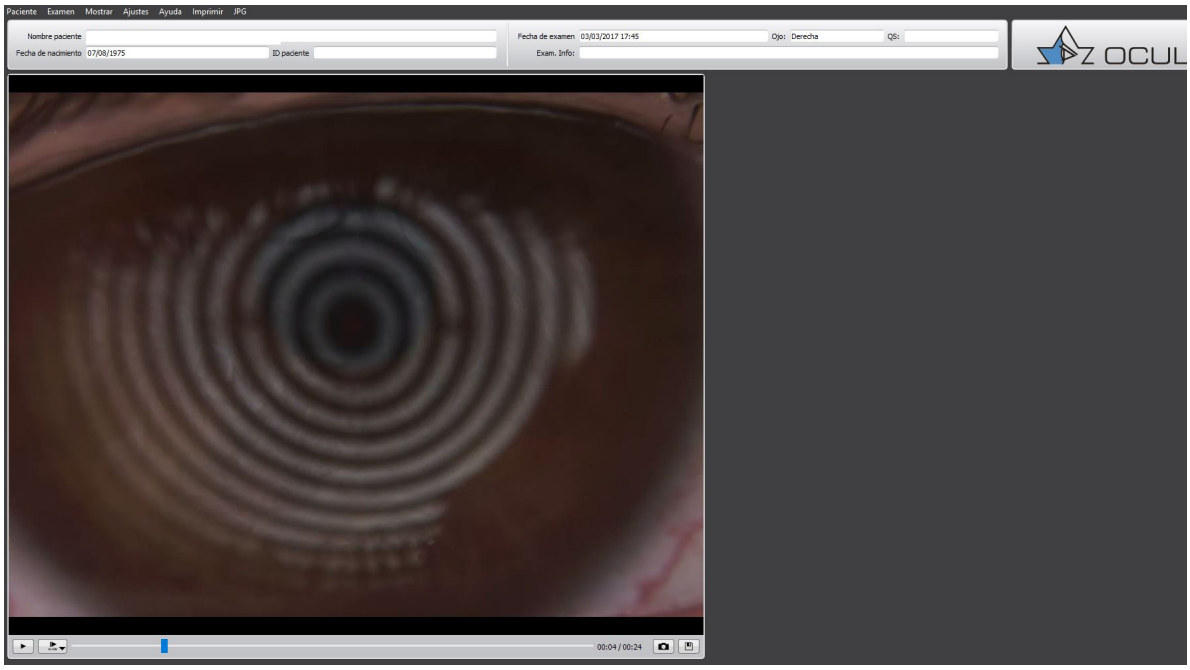


Figure 21. Representative output of the LLP assessment performed with the K5M.

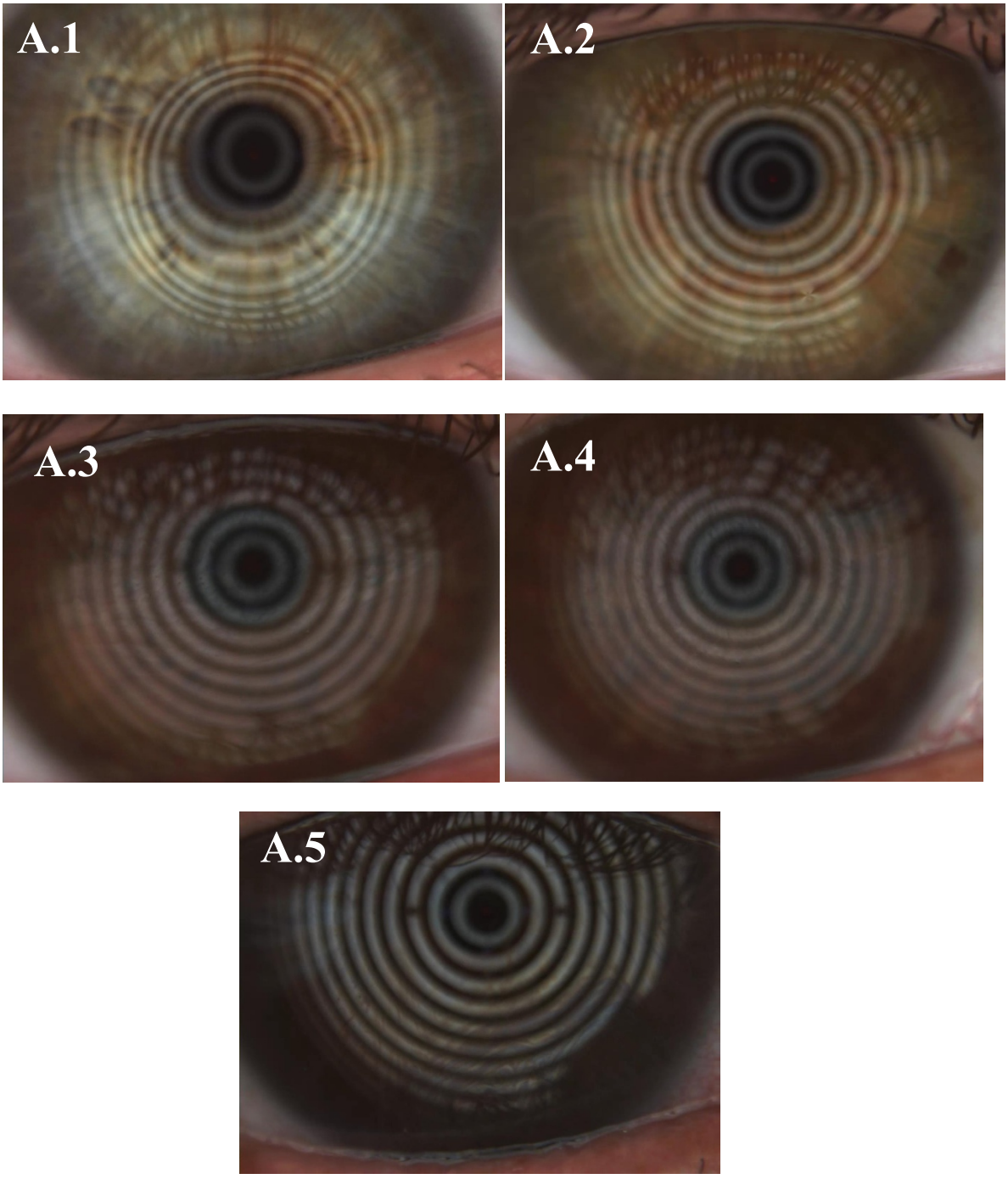


Figure 22. Examples of different LLP observed with the white lamp incorporated in the K5M: **A.1)** Not visible: 13-30 nm, **A.2)** Meshwork: 30-50 nm, **A.3)** Wave: 50-80 nm, **A.4)** Amorphous: 80-90 nm and **A.5)** Colours: 90-180 nm.

2.1.4.4 Tear Film Stability

During tear film stability measurement, participants were instructed to look at the target and blink twice normally. After the second blink, they supressed the blink for as long as they could. The K5M software detected the break-up areas and measured the NIKBUT-first and NIKBUT-avg automatically. Three measurements were obtained, and the time values were averaged for the analysis. (**Figure 23 and Figure 24**)

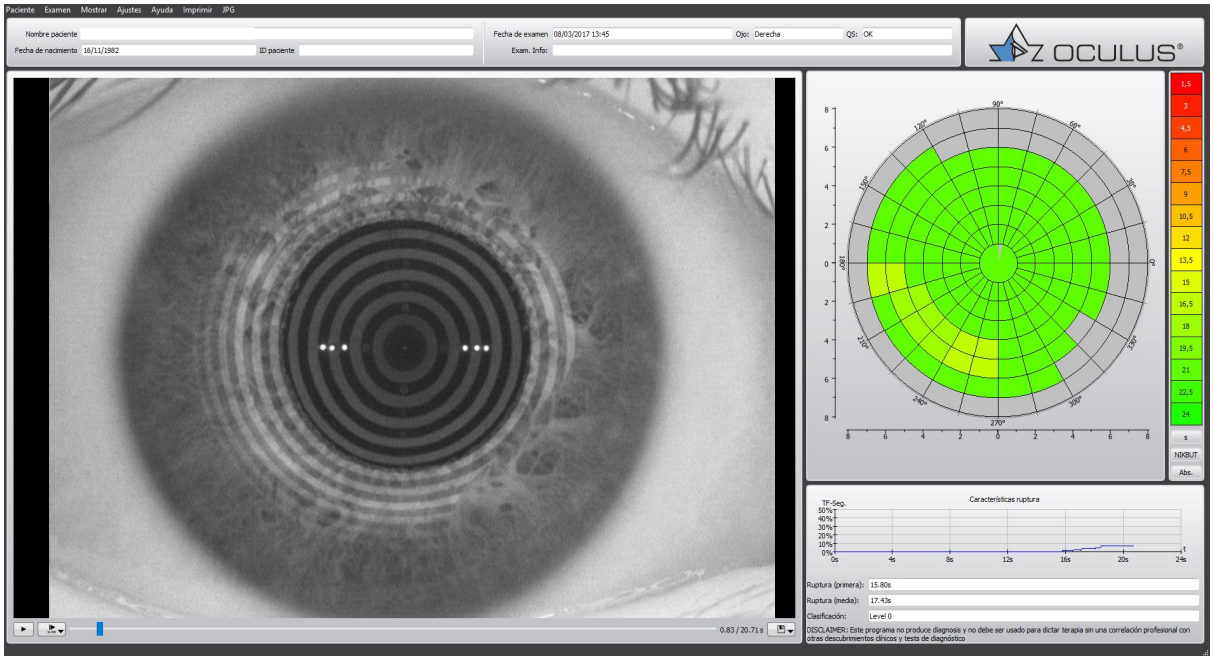


Figure 23. Representative output of tear film stability performed with the K5M. Color-coded map, break-up characteristics map, and classification are included in the software analysis.

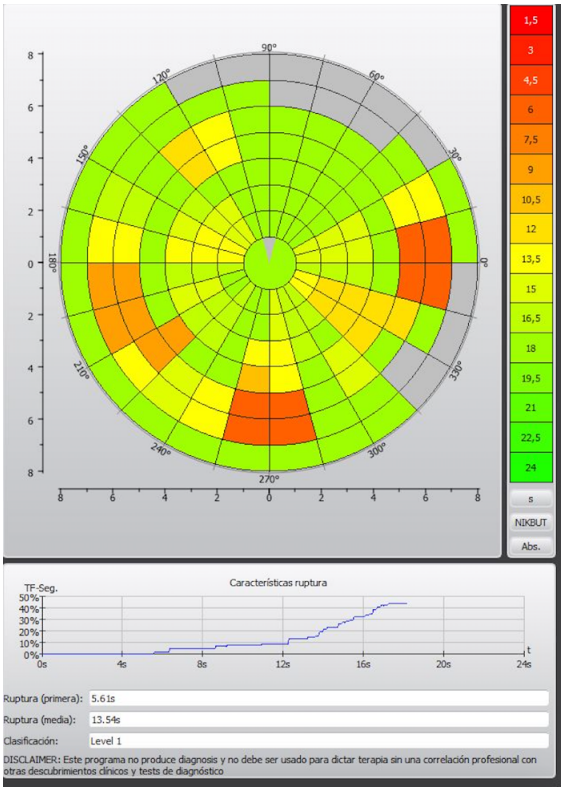


Figure 24. Color-coded map of the NIKBUT measurements during the recording.

2.1.5 Infrared Thermography

An IR thermography camera (FLIR A325; FLIR Systems Inc., USA) was used to register the temperature of the ocular surface (**Figure 25A**). This thermal camera has an image resolution of $320x240$ pixels, a sensitivity of 50 mK and an accuracy of $\pm 2\%$. It can detect temperatures that range from $-20°C$ to $120°C$. Data acquisitions were done with a frame rate of 30 Hz and tear emissivity was set to 0.98 [276].

The camera incorporates a macro-lens in order to obtain a clear image of the eye and it was aligned with respect to the GCC. The participants were instructed to maintain a stable head position on the chin- and forehead rest, close their eyes for 3 seconds and afterwards, open and blink naturally for 40 seconds looking straight (**Figure 26A**). This procedure was established with the purpose of assessing the tear film during the natural blinking of each participant. The body temperature for all participants was measured using a digital thermometer (Citizen Digital Thermometer CTA303, Citizen System,

Japan) and subjects with body temperature $\geq 37\,^{\circ}C$ were excluded. The air-conditioning system was used to maintain both temperature and humidity stable that were registered using a portable weather station (ThermoPro TP-50, Thermo Hygrometre, USA) ($22 \pm 2\,^{\circ}C$ and $30 \pm 5\%$, respectively) **(Figure 26B).** Participants were adapted to the room at least for 20 minutes [273] before the evaluation in order to stabilize the eye temperature and minimize the experimental error.

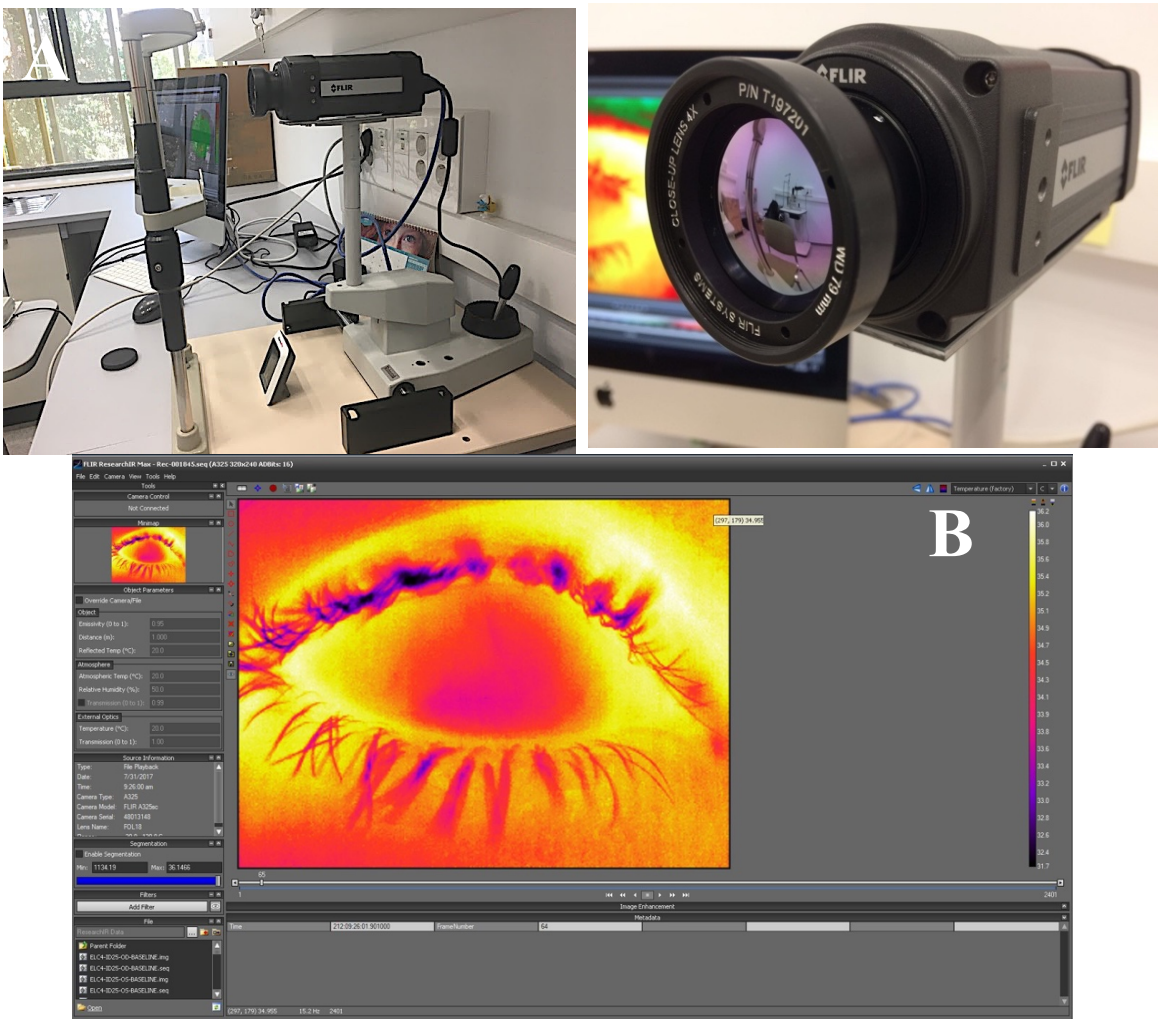


Figure 25. A) Non-contact IR thermography camera and macro-lens integrated. **B)** Representative output of ResearchIR software showing the thermal data obtained from the eye.

The thermal recording was performed in a control group and a group with DED. The inclusion criteria established for the DED subjects were the diagnostic criteria described previously in the DEWS II [30] : subjects must show an OSDI score ≥ 13 and present at

least one sign regarding homeostasis markers (tear film stability, TFO and ocular surface staining). To diagnose the subtype of DED according to the DEWS II, the MGD features, lipid dynamics and tear volume was assessed. The control group was composed of subjects who reported no subjective DED symptoms and lacked DED signs.

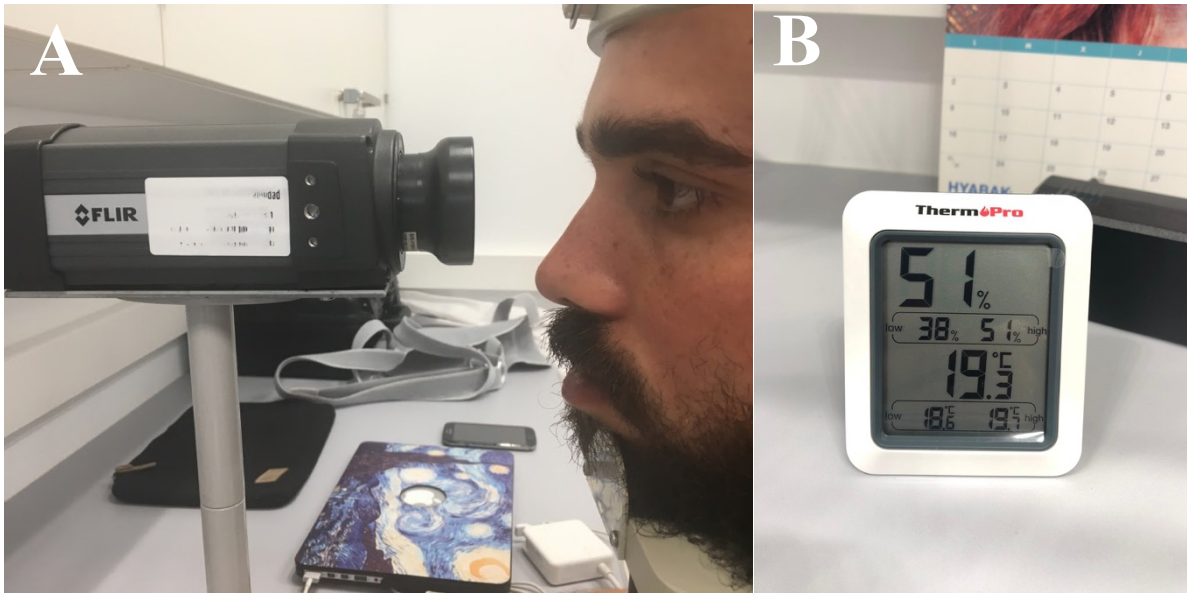


Figure 26. A) Participant undergoing the thermal measurement with the non-contact IR camera. **B)** Weather station used to obtain the humidity and the ambient temperature of the room.

In order to obtain the different OST metrics, the software which provided by the manufacturer was used **(Figure 25B).** Next, the temperature data of the sequences were exported and analysed off line to extract metrics of interest. Those frames affected by blinks were detected and removed for the following analysis. The region of interest (ROI) was manually determined by a single examiner and kept constant for each sequence (**Figure 27**).

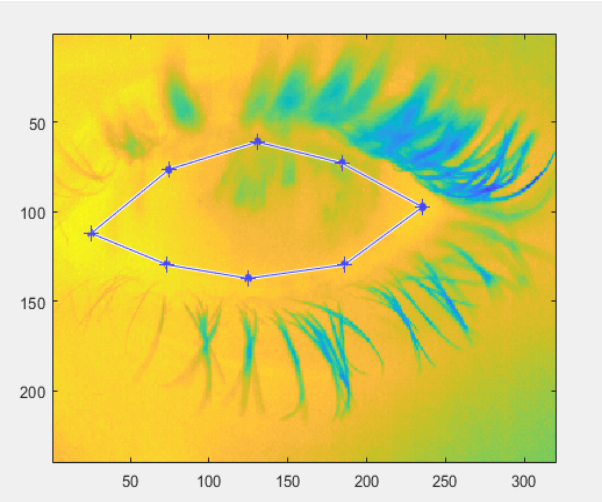


Figure 27. ROI marking on ocular surface in order to obtain OST metrics with the custom algorithm.

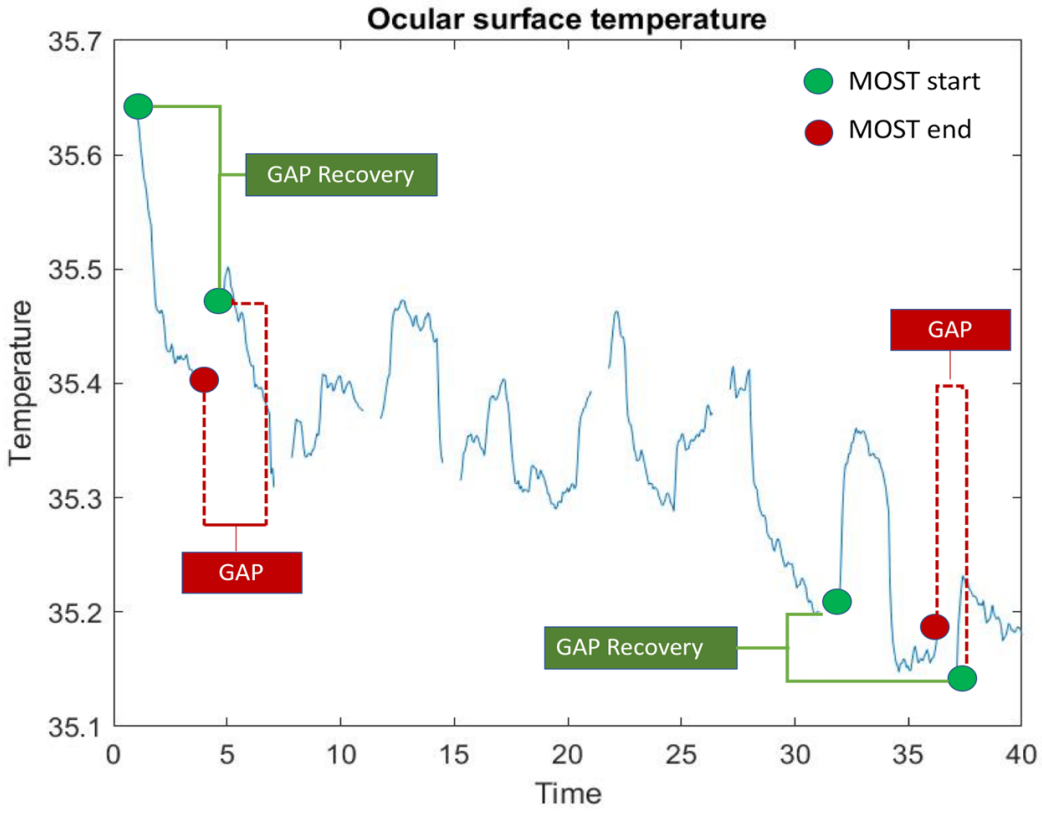
A custom algorithm developed in Matlab (MathWorks Inc., Natick, USA) by *Clara Llorens Quintana* (EDEN fellow in the Department of Biomedical Engineering, Wroclaw University of Science and Technology, Wroclaw, Poland) and was used to analyse OST data. The TER was computed according to the approach proposed by Tan et al.[266].

3.1.5. 1 Ocular Surface Temperature (OST) metrics

In order to characterize the thermal changes, several OST metrics were generated after image analysis. Because the established the OST recording process was established based on the natural blinking, the first and the last complete IBIs were taken in order to study the temporal OST changes. As reported by Johnston et al. [271], the IBI assessment can reveal a substantial amount of information for a given sample compare to the blink rate. In this study, the first IBI was chosen in order to assess the OST just after opening the eye and the last complete IBI in order to assess the OST after a period of time. Six OST metrics were generated as shown in **Table 3.**

Table 3. The six OST metrics studied.

OST metrics	Description
TER	Tear Evaporation Rate (g/cm^2/s)
MOST	Mean OST of the ROI (°C).
GAP	OST difference between the end of one IBI and the beginning of the next IBI (°C). (**Figure 28**)
GAP recovery	OST difference between the beginnings of two consecutive IBIs in the ROI (°C). (**Figure 28**)
MIN	Minimum temperature in the ROI (°C).
MAX	Maximum temperature in the ROI (°C).



MOST: Mean OST of the ROI (°C). **GAP:** OST difference between the end of one IBI and the beginning of the next IBI (°C). **GAP recovery**: OST difference between the beginning of two consecutive IBIs in the ROI.

Figure 28. Example of OST output generated by the custom algorithm. The illustration represents the OST metrics GAP (*red*) and the GAP recovery (*green*), respectively.

2.1.6 Slit-Lamp Examination

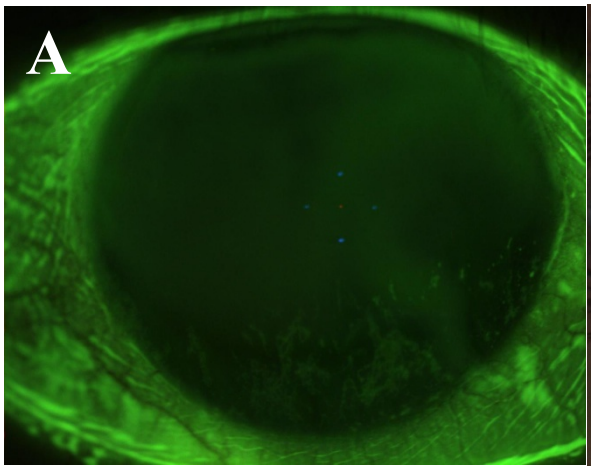
The ocular surface examination was performed with a slit-lamp biomicroscopy (Topcon SL-D4, Tokyo, Japan). The cornea, conjunctiva and adnexa were observed under diffuse illumination using ×10 – ×16 magnification **(Figure 29).**

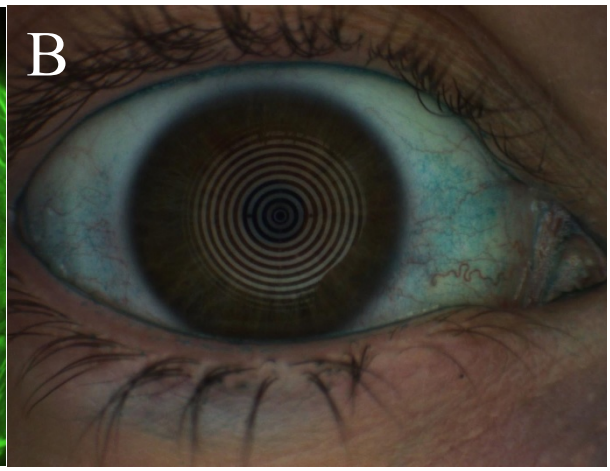
Figure 29. Ocular surface assessment performed by the researcher using the slit-lamp biomicroscopy.

Corneal integrity was assessed by instilling fluorescein in the eye using a fluorescein strip (Tiedra Laboratories, Madrid, Spain) that was previously wetted with saline solution (Saline solution, AVIZOR, Madrid, Spain) (**Figure 30**). Two minutes after the instillation, the corneal staining was graded using the Oxford scoring scheme [124] (0-5 score; **Figure 31A and 31C**). The evaluation was performed using a cobalt blue filter incorporated in the illumination system together with a yellow Wratten #12 filter. Conjunctival integrity was assessed under diffuse white illumination using lissamine green strips (Tiedra Laboratories, Madrid, Spain) and graded using the Oxford scoring scheme (**Figure 31B and 31C**).

Figure 30. Clinical set used to perform the ocular surface examination (saline solution, topical anaesthesia, fluorescein and lissamine green strips and Schirmer strip, respectively).

A few minutes before the corneal staining assessment, the TBUT was assessed asking the participants to blink a couple of times and then to maintain their eyes open. The appearance of the first dry spot considered as the TBUT, it was measured three times with a stopwatch and averaged in order to obtain a reliable value.





C

Staining appearnace	Grade	Verbal descriptor
	0	Absent
	I	Minimal
	II	Mild
	III	Moderate
	IV	Marked
> IV	V	Severe

Figure 31. A) Inferior corneal staining observed with fluorescein dye **B)** Conjunctival staining with lissamine green **C)** Oxford scoring scheme (0-5 scores) used for corneal and conjunctival staining grading.

2.1.6.1 Eyelid Features

The examination of the eyelids was performed under diffuse white illumination using ×10 – ×16 magnification. Before fluorescein instillation, lid abnormalities and MG grading were observed and scored according to Foulks/Bron scoring [181] as recommended by the *Diagnosis Subcommittee from International Workshop on Meibomian Gland Dysfunction* [166]. Lid margin abnormality scores from both eyelids were 0 (absent) or 1 (present) for the following parameters: irregularity of the lid margin, lid margin telangiectasia, lash loss, trichiasis, malposition, plugging, capping, orifice narrowing, orifice vascular invasion, loss of cuffing definition and foam secretion. The eyelid margin thickness was assessed on a scale from 1 to 5: 1–2=thin; 3=normal; 4–5= thick. The meibum quality from the central 8 MG was assessed on a scale from 0 to 3: 0 = clear meibum readily expressed; 1= cloudy meibum expressed with mild pressure; 2= cloudy meibum expressed with more than moderate pressure; 3= meibum could not be expressed even with strong pressure. The number of functional MGs was assessed on a scale from 0 to 3: 0= more than 5 glands expressible; 1= 3 to 4 glands expressible; 2 = 1 to 2 glands expressible; 3 = no glands expressible. Lid wiper epitheliopathy (LWE) of the UL and LL was assessed using a combination of fluorescein and lissamine green (Korb Protocol B). The higher of the final fluorescein or lissamine green staining were used as LWE severity grade (0 = absent, 1 = mild, 2 = moderate and 3 = severe) [277] (**Figure 32**).

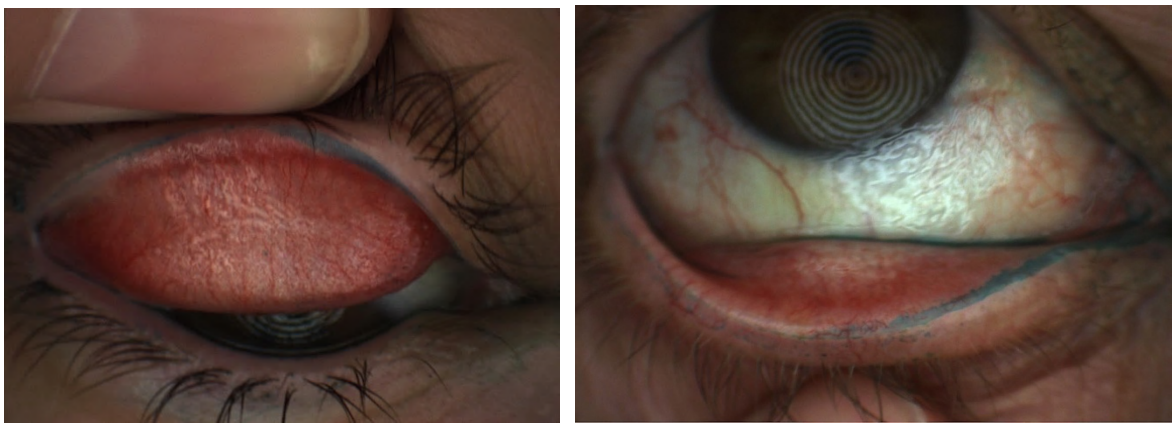


Figure 32. Lid wiper epitheliopathy of the UL and LL observed with lissamine green.

2.1.7 Non-contact Infrared Meibography

NIM was performed to observe MG structure from the UL and LL of each participant (**Figure 33**). UL and LL of each participant were everted carefully and IR images were obtained using the infrared camera integrated in the K5M. Afterwards, MGL of the UL and LL was graded subjectively using the *meiboscore* (grade 0 = no gland loss; grade 1 = area of gland loss <33%; grade 2 = area of gland loss 33%–67%; and grade 3= area of gland loss >67%) [178]. The *meiboscore* for each eyelid was summed to give a total score of 0 to 6 (**Figure 34**).

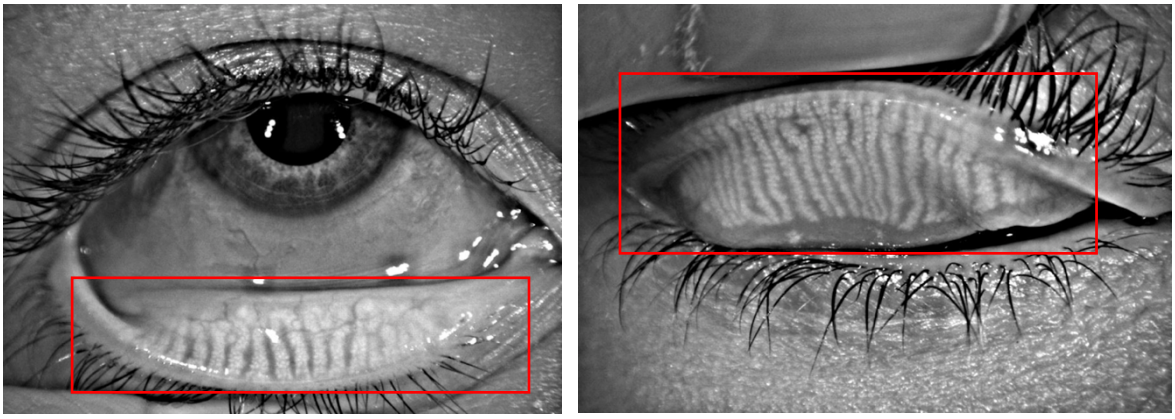


Figure 33. Meibography images from the LL and UL of a normal participant.

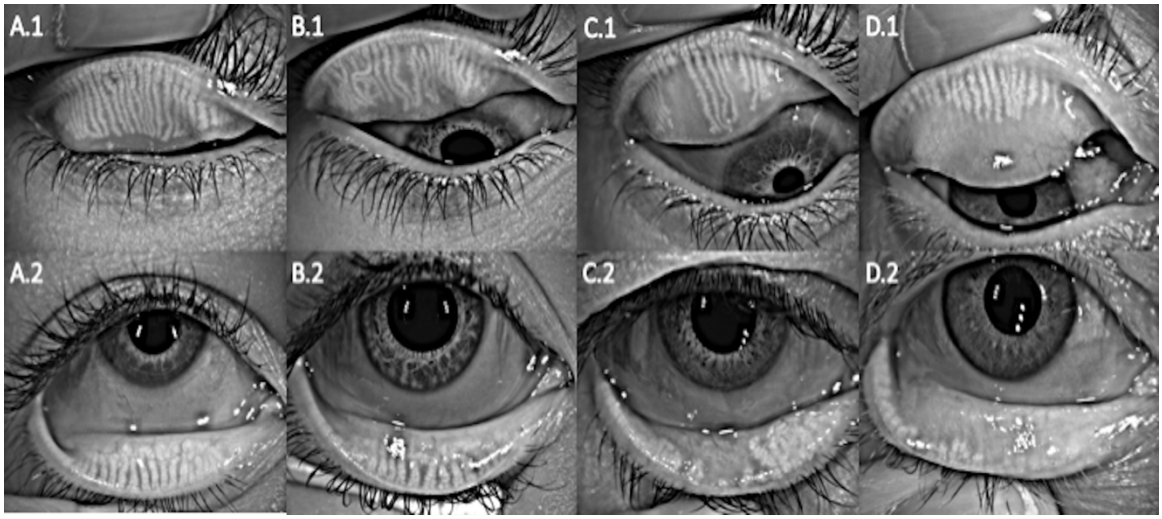


Figure 34. Different grades of MGL in the UL and LL obtained by NIM (K5M). **A.1** and **A.2)** Participants without MGL in the UL and LL (total meiboscore 0; = 0%). **B.1** and **B.2)** Participants with slight MGL in the UL and LL (total meiboscore 1; < 33%). **C.1** and **C.2)** Participants with moderate MGL in the UL and LL (total meiboscore 2; 33-66%). **D.1** and **D.2)** Participant with severe MGL in the UL and LL (total meiboscore 3; >66%).

2.1.8 Tear Film Volume

Schirmer's test was carried out as the final test and performed with topical anaesthesia (Colirio Anestésico Doble®, Alcon Laboratories, Spain). One drop of topical anaesthesia was instilled on the conjunctival lower fornix of the chosen eye, five minutes prior to the test. Afterwards, the Schirmer strip (35-mm Whatman filter paper; Tiedra Laboratories, Spain) was placed into the lower conjunctival sac at the junction of the lateral and middle thirds (avoiding contact with the cornea) and the length of wetting was recorded after 5 minutes **(Figure 35B).** Participants were seated at rest and their eyes closed during the test (**Figure 35A**).

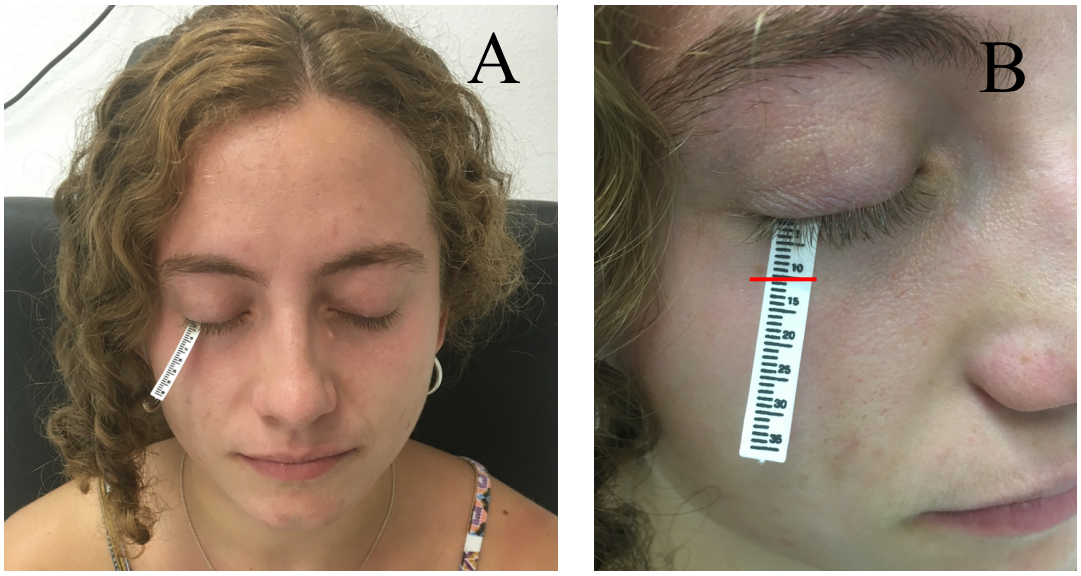


Figure 35. (A) A participant performing Schirmer test **(B)** Schirmer test strip wetting during measurement (the *red line* indicates the wetting measurement).

2.1.9 Symptomatology Assessment

During the clinical examination, participants were required to complete five of the most common DED questionnaires used in the clinical setting: OSDI [72], MQ[83], SPEED[82], SANDE[76] and the DEQ-5[78] (**Annex 4**).

In order to ensure the reliability of the information obtained by DED questionnaires and also to avoid interference between measurements, the questionnaires were completed by the participants throughout the examination, randomly. For example,

some of the questionnaires were performed at the end of K5M measurements and before the thermography recording in order to allow tear film to recover from the last measurements (5-10 minutes). Questionnaires were also completed by the participants after the conjunctival staining and before the Schirmer test with the same objective.

2.1.10 Automatic Algorithm for Meibomian Gland Assessment

An automated algorithm that analyses infrared images of the MG using image processing techniques has been developed by *Clara Llorens Quintana* (EDEN fellow in the Department of Biomedical Engineering, Wroclaw University of Science and Technology, Wroclaw, Poland) and used to obtain objective information about the MG morphology (**Figure 36** and **Annex 6**). The morphometric parameters obtained were: MGL, gland length, gland width and gland irregularity (also named as *tortuosity or MG distortion*) extracted from a total of 149 meibography images (out of 161) from the everted UL of the present study.

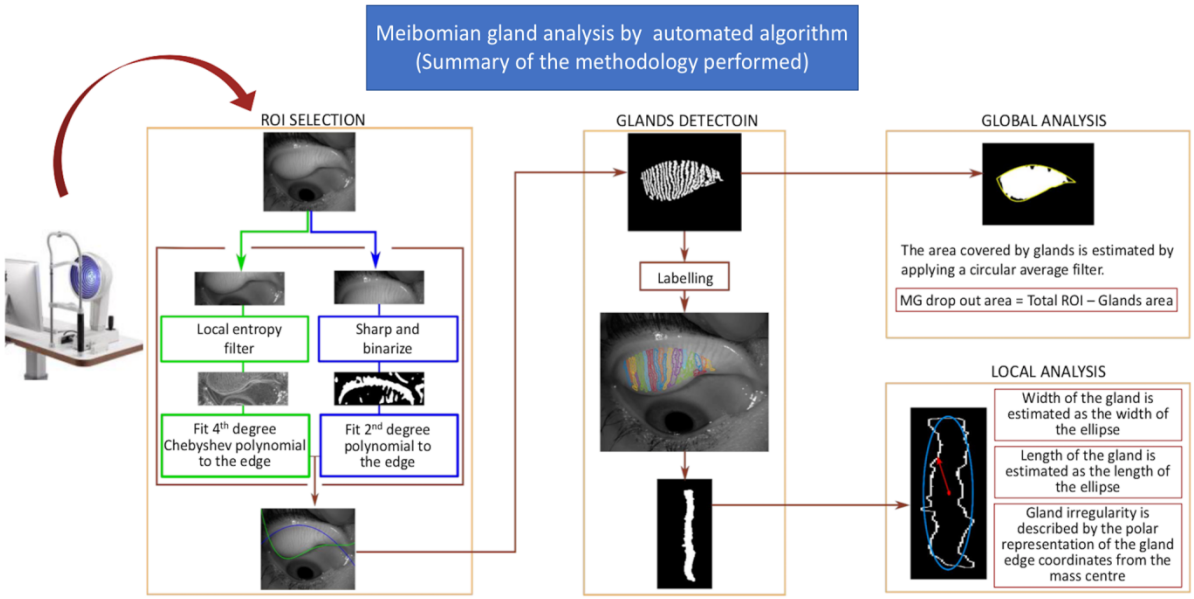


Figure 36. Summary of the methodology performed for meibomian gland analysis. More detailed in **Annex 6** (Image courtesy of *Clara Llorens Quintana, ESR fellow*).

Automated and objective analysis of the glands was carried out in a batch mode with no user input. Manual adjustment consisting of ROI selection was necessary in 9 of the images (6.04%). In 6 of those cases, the images were not acquired properly while in the remaining 3 images, a pre-processing problem was encountered. Acquisition problem can be due to an unfocused image, off center image with part of the tarsal conjunctiva out of the frame or because the lower boundary of the upper eyelid is attached to the lower eyelid (**Figure 37**). Despite this, it is worth to mention that the algorithm never failed in the steps after the ROI selection.

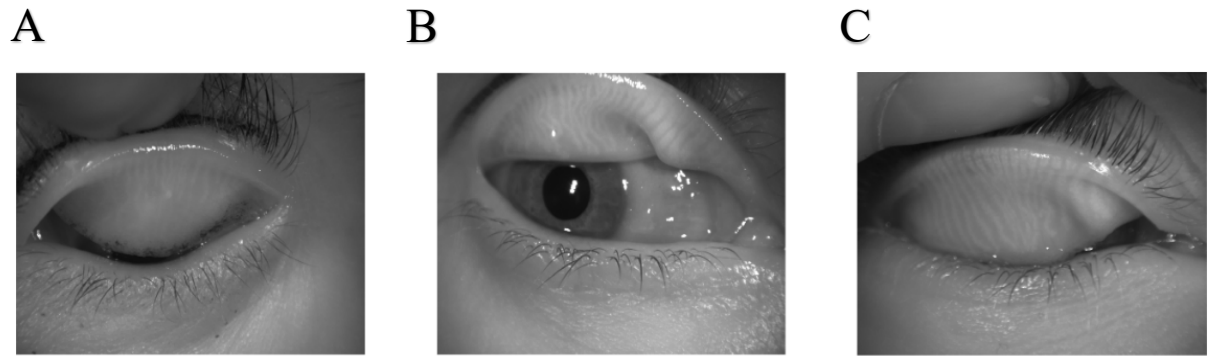


Figure 37. Example of three images where the algorithm was unable to detect the correct ROI due to a problem during the acquisition **A)** unfocused image (focus is at the eyelashes), **B)** out of frame image and **C)** image where the upper eyelid is attached to the lower eyelid. (Image courtesy of *Clara Llorens Quintana, ESR fellow*).

2.1.11 Statistical Analysis

2.1.11.1 Sample Size Calculation.

The study evaluates numerous parameters related to the lacrimal function, then a correct estimation of the sample size is considered several measured parameters measured (thus obtaining an *n* for each parameter studied), to consider the highest value of n for the study, since this will achieve a greater statistical power. The calculations made for the sample size estimation make use of the expression of a normal distribution with a level of statistical significance of 5% $(\alpha = 0.05)$, the minimum sample size necessary to reach a statistical power of 80% $(1 - \beta = 0.8)$. For the calculation of the sample size it is necessary to establish the minimum detectable change (or clinically significant difference) in the variable to be studied, as well as the expected standard deviation. These two variables can be set by some criterion or extracted from the published literature on the subject studied.

Meibography:

Based on the qualitative analysis of the meibography images, the grading scale for MGL assessment consist of 4 degrees, then the minimum clinically detectable change using this scale would be 1 degree. From the study carried out by Heiko and Britta [188], 0.9 can be considered as the standard deviation for meibography.

The minimum sample size necessary for this data is $\boldsymbol{n = 14.}$

TBUT:

Considering the data from the research work performed by Paschides et al.[278], TBUT = 13 seconds can be considered normal for subjects, and TBUT = 7 seconds for subjects with DED, then the difference between groups would be 6 seconds. In addition, this study has reported a standard deviation of 6 seconds.

Regarding the data provided, the minimum sample size necessary is $\boldsymbol{n = 17}$

Schirmer test:

From the study conducted by Kashkouli et al.[279], the Schirmer value for normal subjects was 26.76 mm in 5 minutes and 13.15 mm for DED subjects, considering 16.61 mm as the significant change. In addition, the same study reported 16.7 mm as standard deviation of Schirmer test.

According to data provided, the minimum sample size necessary is $\boldsymbol{n = 14}$

After analysing each variable, it was finally decided to set $n \geq 17$ *in each group to achieve a correct statistical power for this study.*

2.1.11.2 General Statistical Analysis Description.

Statistical analysis was performed using (SPSS Version 25; SPSS, Chicago, IL). Normality of the data distribution was tested using the Kolmogorov–Smirnov test and was rejected for $p < 0.05$. Paired samples t-test (if normality of the data was assumed) and Wilcoxon test (if normality of the data was rejected) were used. Also, independent t-test (if normality of the data was assumed) and Mann-Whitney test (if normality of the data was rejected) were used. Post hoc tests were performed for multiple comparisons (Duncan's Test for ANOVA and Bonferroni for Kruskal- Wallis). Correlations among variables were assessed through Pearson and Spearman coefficients. The correlations were considered strong if > 0.80, moderately strong if between 0.5 and 0.8, fair within the range of 0.3 and 0.5 and poor if < 0.30 [280]. The values are expressed as mean±SD/ median (IQR). The significance level was set $p < 0.05$ with $> 95\%$ of confidence level.

Additionally, in the second study, Cohen's kappa coefficient (weighted kappa value- 95% of confidence) was calculated and classified as follow: 0.00 (poor), $0.00 - 0.20$ (slight), $0.21 - 0.40$ (fair), $0.41 - 0.60$ (moderate),$0.61 - 0.80$ (substantial) and $0.80 - 1.0$ (close to perfect) [281]. Associations between incidence of changes in the eyelid and MGL were assessed using Chi-squared ($\chi 2$).

In addition, In the third study, in order to classify the irregularity in groups according to the degree of irregularity (*low, medium and high*), the data has been clustered in three classes and their intra-class variance was minimal using Otsu's classification algorithm [282].

Chapter 3

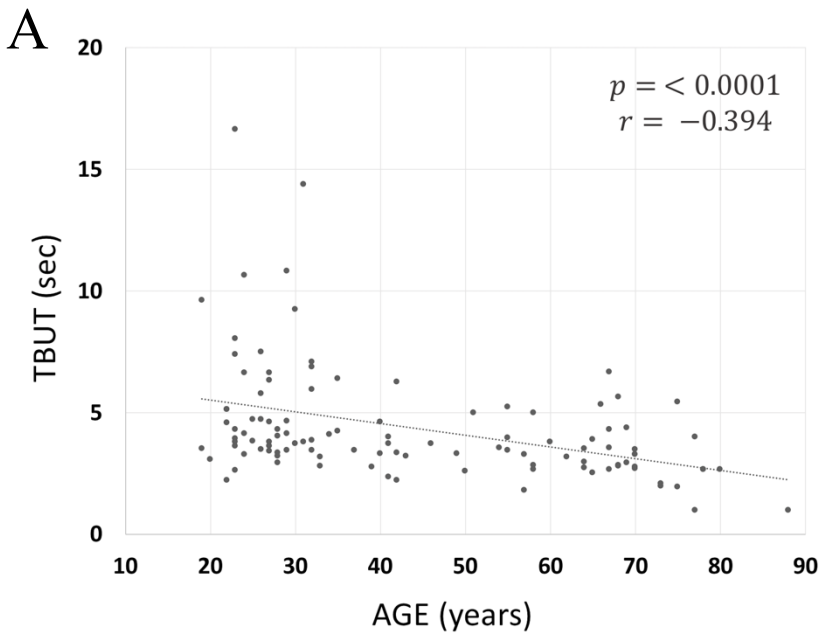
Results

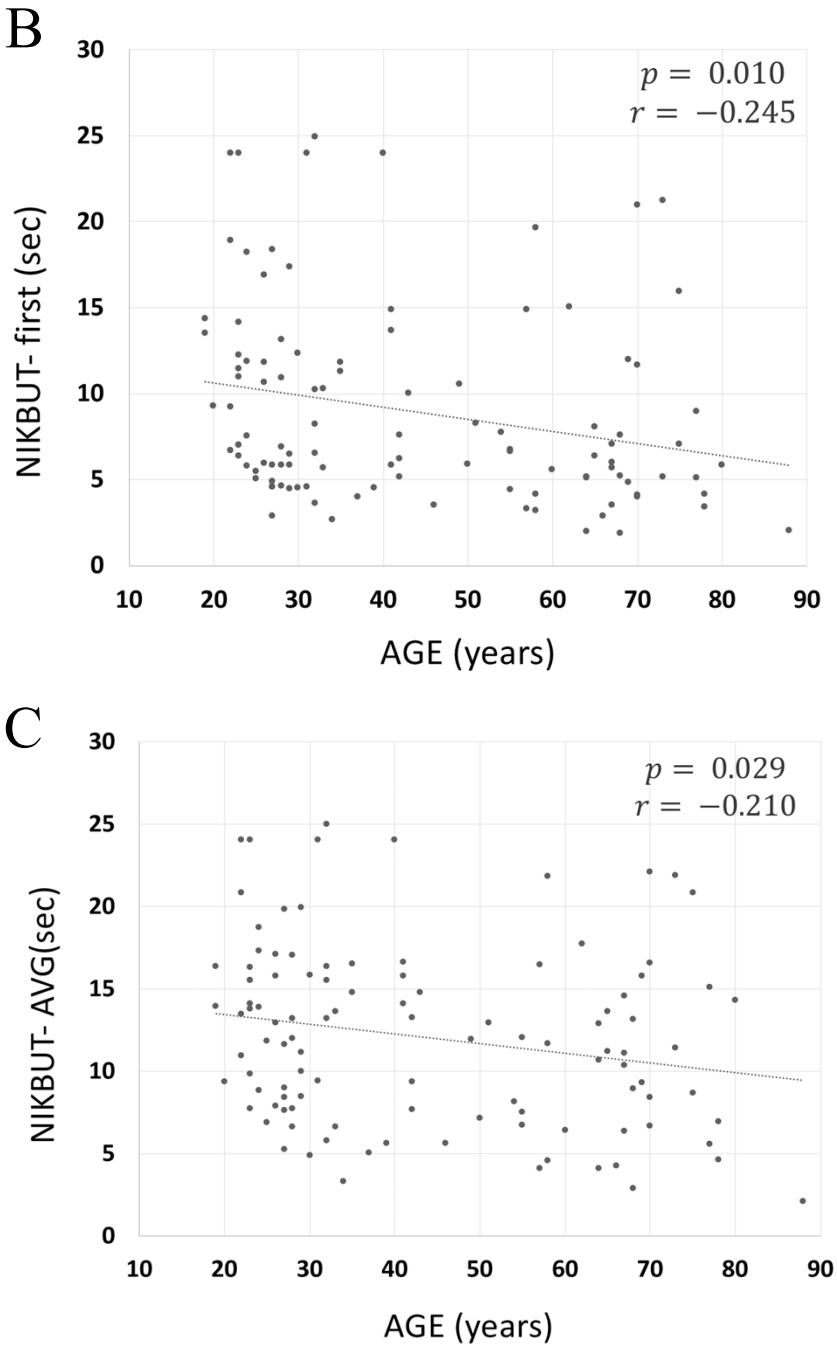
Study I

The effect of ageing on the ocular surface parameters

A total of 110 participants were enrolled in the study (70 females and 40 males). The mean age of the participants was 44 ± 19 years (ranging from 19 to 88 years).

A negative correlation was observed between TBUT and NIKBUT (first and average) with age (fair; $r = -0.394, p < 0.0001$; poor; $r = -0.245, p = 0.010$; poor; $r = -0.210, p = 0.029$, respectively) (see **Figures 38A, 38B and 38C**).

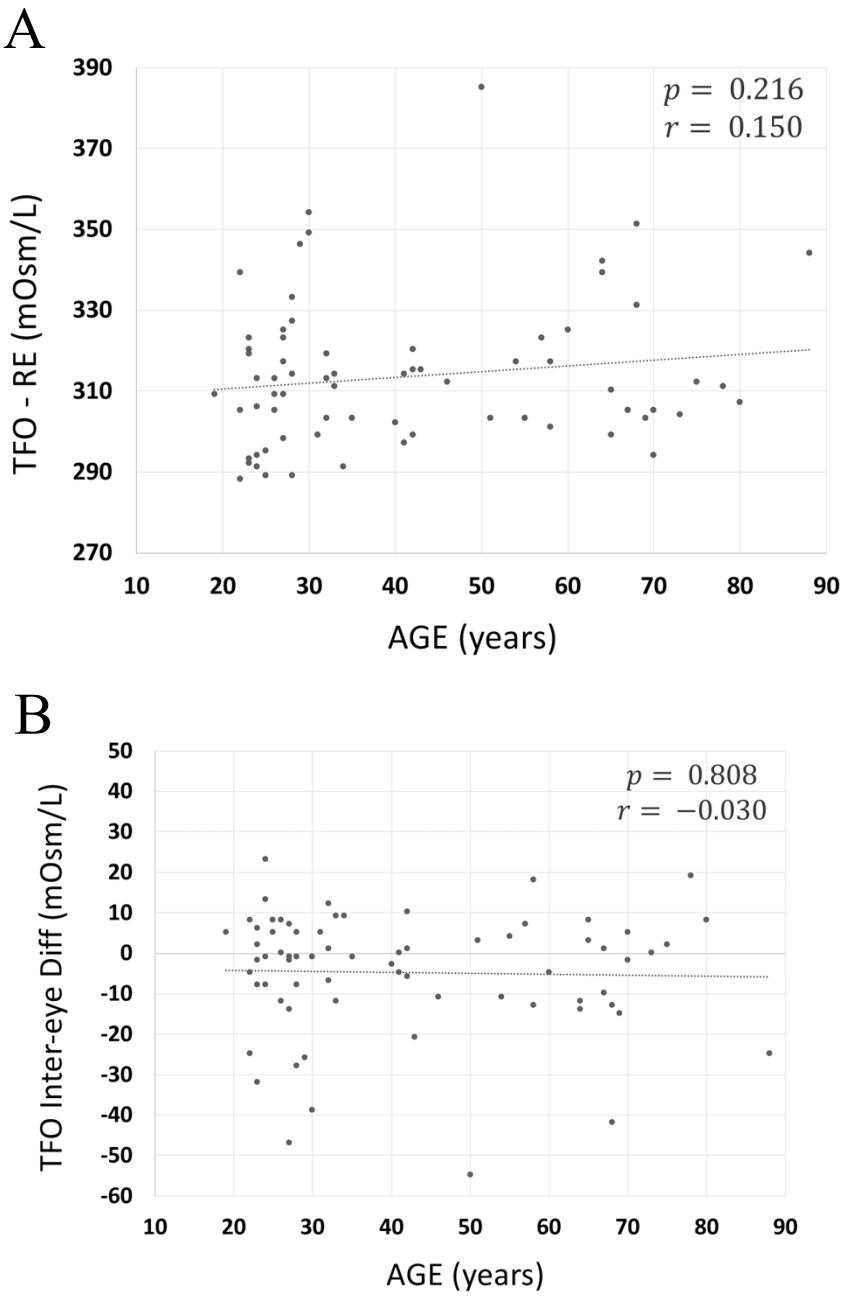




TBUT: Tear break-up time(seconds); NIKBUT-first: first rupture non-invasive Keratograph tear film break-up time (seconds); NIKBUT-avg: average of non-invasive Keratograph tear film break-up time (seconds).

Figure 38. Correlations between Age and **A)** TBUT measured using fluorescein dye; **B)** NIKBUT-first measured with the K5M and **C)** NIKBUT-avg measured with the K5M.
(r, Pearson correlation coefficient).

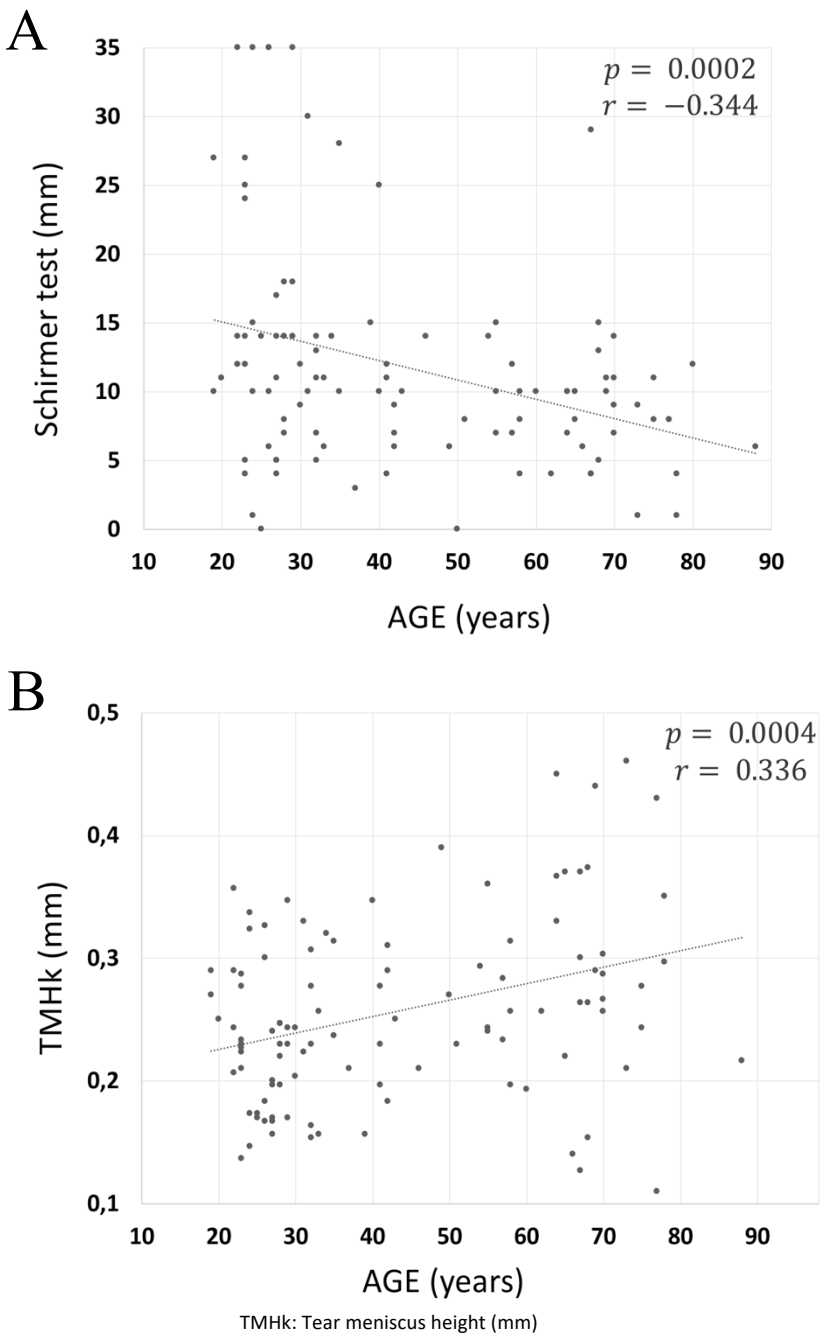
As it is shown in **Figures 39A and 39B**, TFO from RE and TFO inter-eye difference showed no significant correlation with age (poor; $r = 0.150, p = 0.216$ and poor; $r = -0.030, p = 0.808$, respectively).



TFO: Tear film osmolarity (mOsms/L) and Inter-eye difference.RE: Right eye

Figure 39. Correlations between Age and **A)** TFO from RE measured with TearLab Osmolarity System **B)** Inter-eye difference.
(r, Pearson correlation coefficient)

Schirmer test (see **Figure 40A**) showed a negative correlation with age (fair; $r = -0.344, p < 0.001$). While TMHk showed a positive correlation with age (fair; $r = 0.336, p < 0.001$) (see **Figure 40B**).



TMHk: Tear meniscus height (mm)

Figure 40. Correlations between Age and **A)** Schirmer test performed with topical anaesthesia and **B)** TMHk measured with the K5M.
(r, Pearson correlation coefficient).

Regarding ocular staining and redness, significant positive correlations were observed between corneal and conjunctival staining score with age (fair; $r = 0.400, p < 0.0001$ and moderately strong; $r = 0.638, p < 0.0001$, respectively) (see **Figures 41A and 41B**) and also between BR and LR with age (moderately strong; $r = 0.619, p < 0.0001$ and $r = 0.659, p < 0.0001$, respectively) (see **Figures 42A and 42B**).

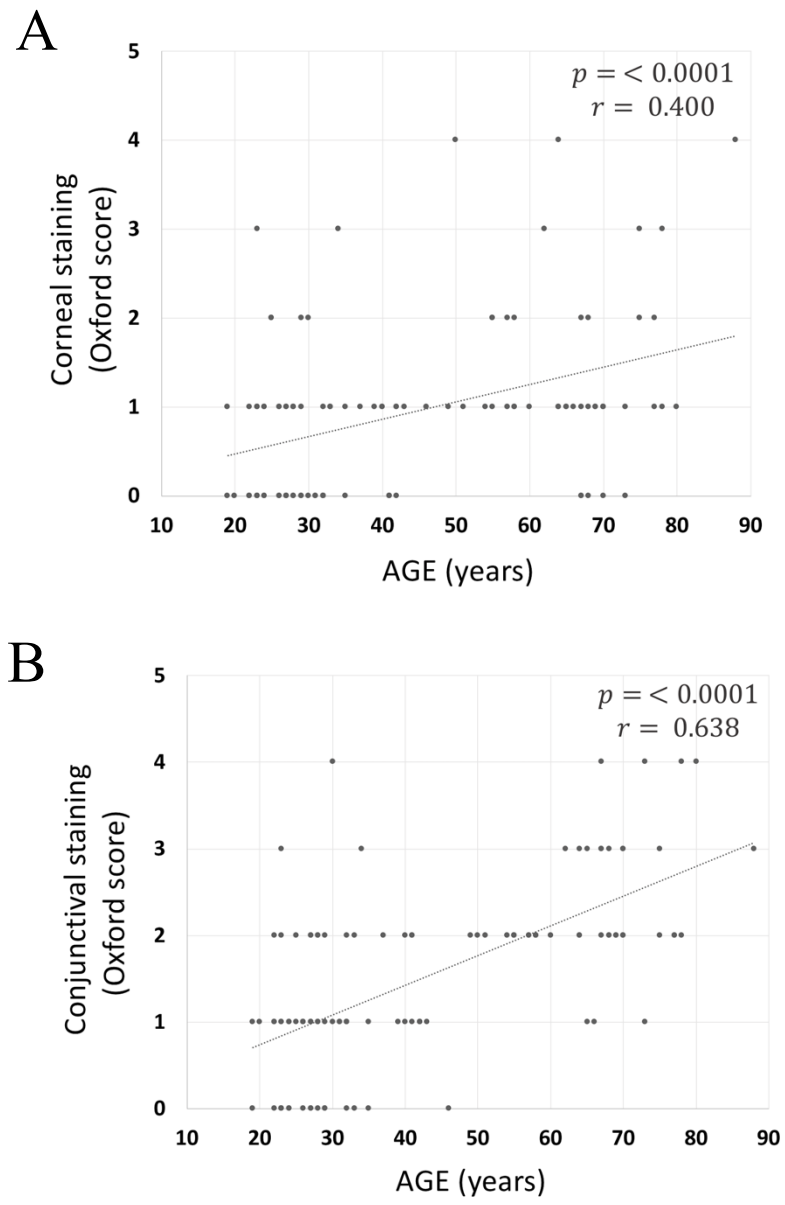


Figure 41. Correlations between Age and **A)** Corneal staining graded the Oxford scoring scheme; **B)** Conjunctival staining graded the Oxford scoring scheme;
(r, Spearman correlation coefficient).

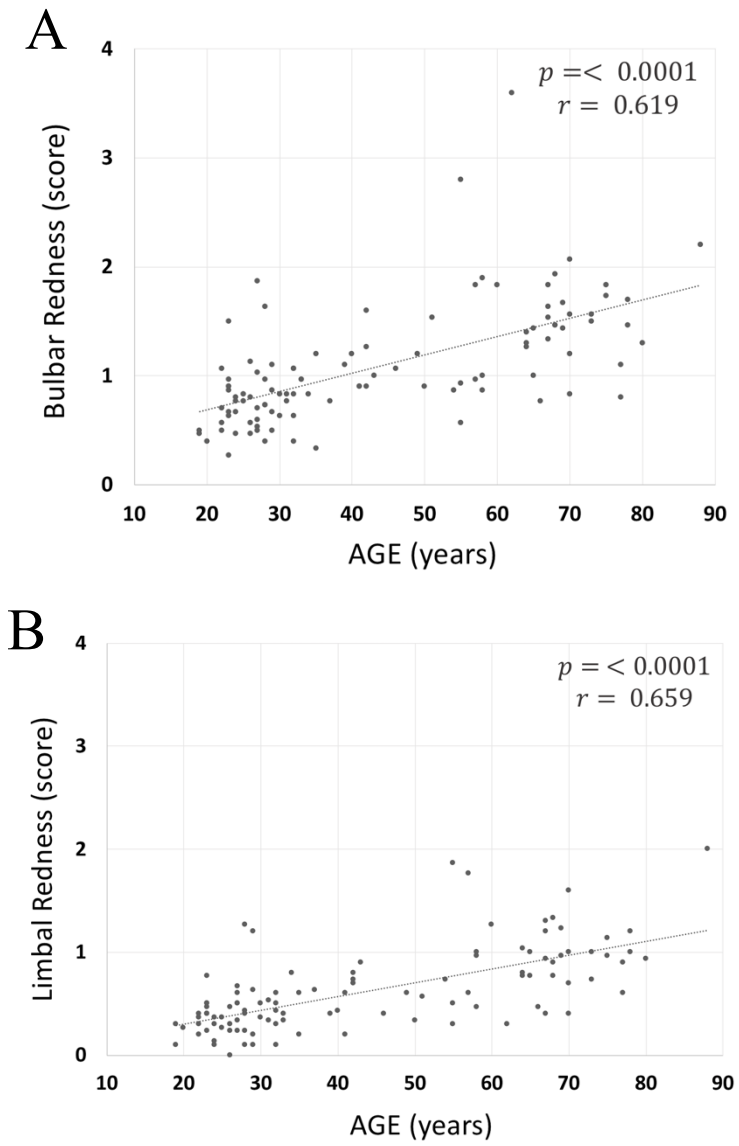
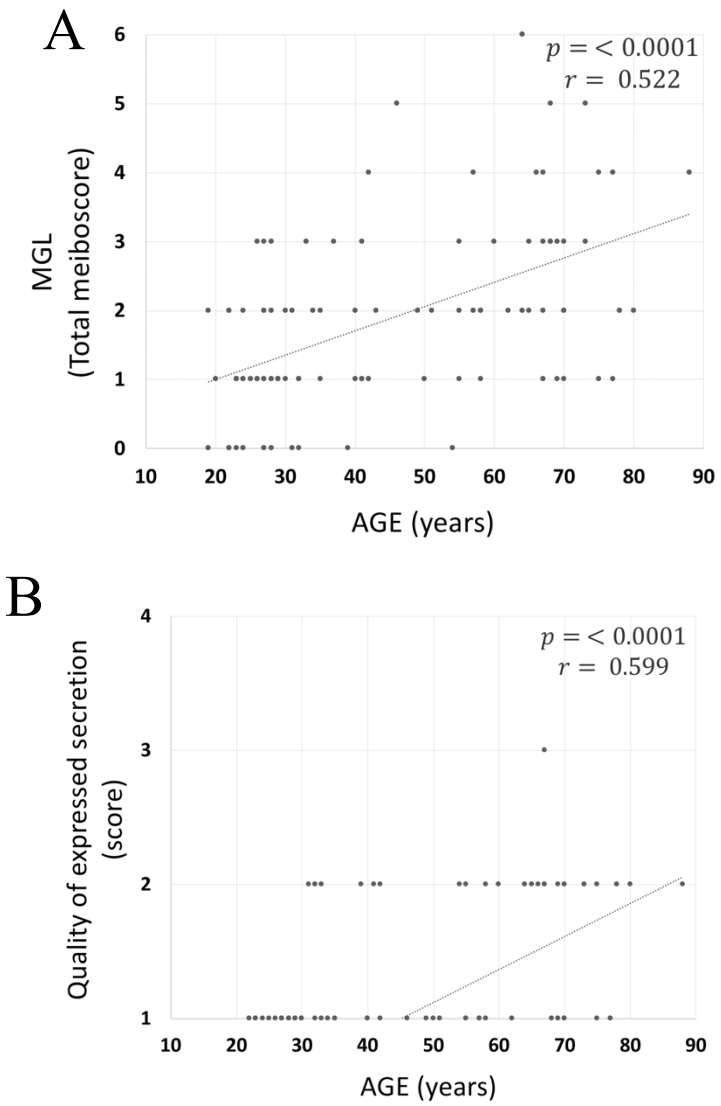


Figure 42. Correlations between Age and **A)** Bulbar Redness measured with the K5M and **B)** Limbal Redness measured with the K5M.
(r, Pearson correlation coefficient).

Figures 43, 44 and 45 show the lid margin and MG features assessed, respectively. Significant correlations were observed between age and every lid margin/MG features (MGL (see **Figure 43A)**, quality of the secretion expressed (see **Figure 43B)**, eyelid margin thickness(see **Figure 44A**), number of functional MG (see **Figure 44B**) and LWE from the UL and LL **(**see **Figure 45A and 45B)**(moderately strong $r = 0.522, p < 0.0001$; $r = 0.599, p < 0.0001$; $r = 0.650, p < 0.0001$; $r = 0.651, p < 0.0001$; fair; $r = 0.305\ p = 0.0015$; and $r = 0.393\ p < 0.0001$; respectively).



MGL: Meibomian gland loss graded with meiboscore scale; NIM: Non-contact meibography; MG: Meibomian glands

Figure 43. Correlations between Age and **A)** MGL observed by NIM; **B)** Quality of expressed MG secretion.
(r, Spearman correlation coefficient).

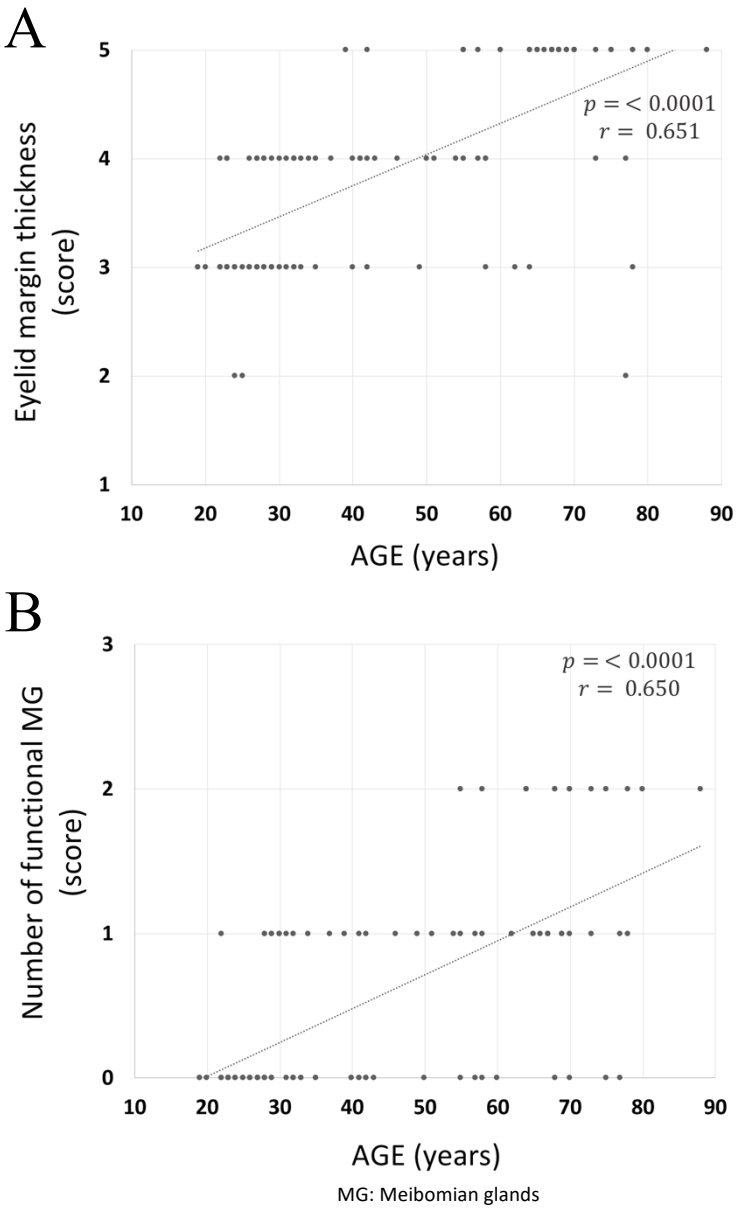
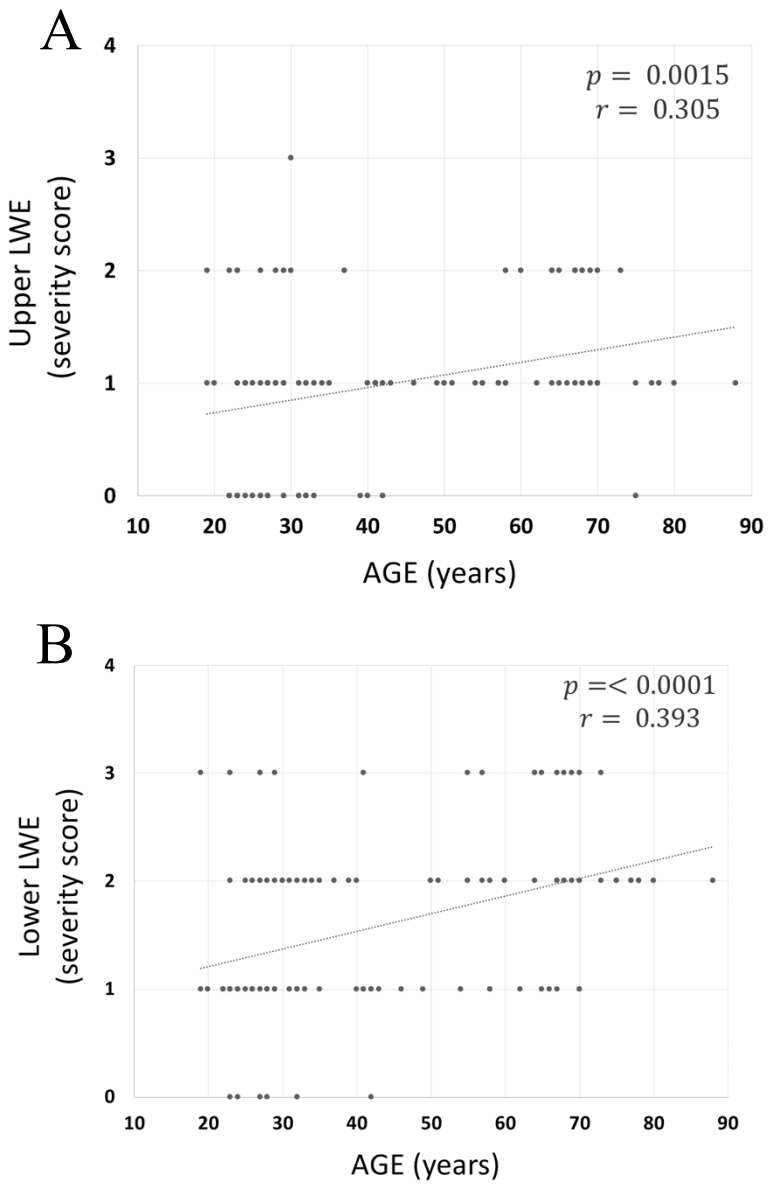


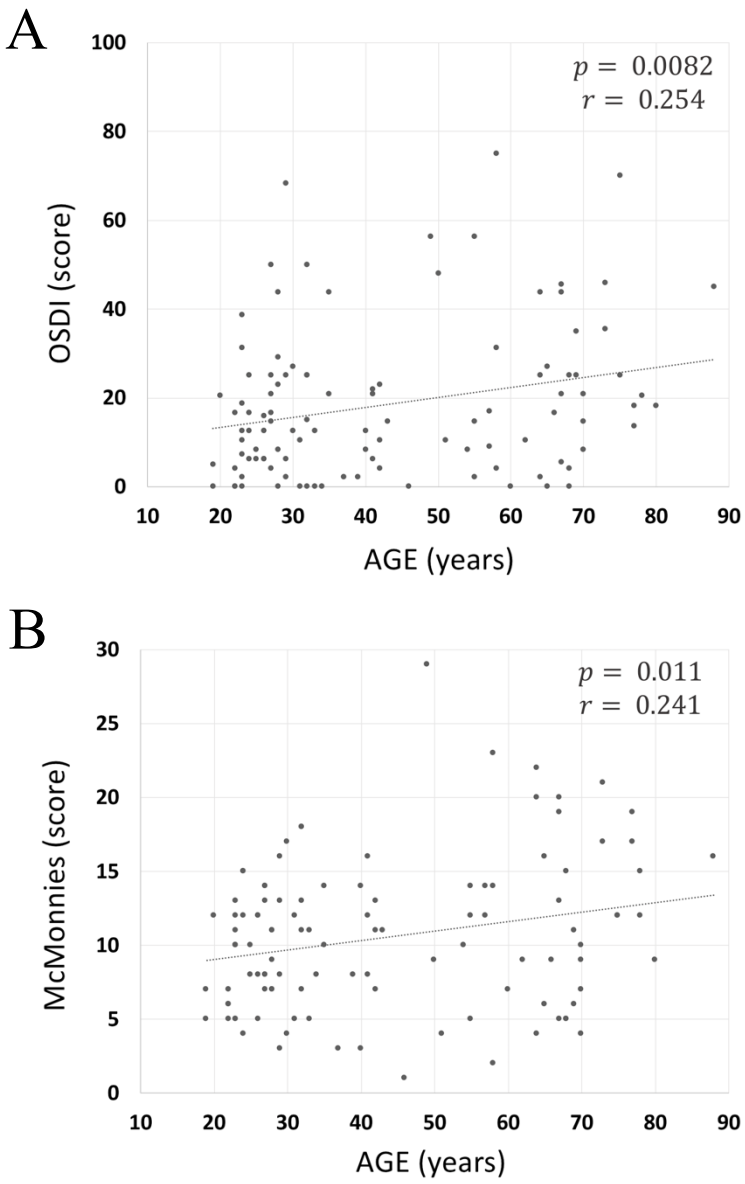
Figure 44. Correlations between Age and **A)** Number of functional MG and **B)** Eyelid margin thickness assessed by slit-lamp.
(r, Spearman correlation coefficient).



LWE: Lid wiper ephiteliopathy (score).

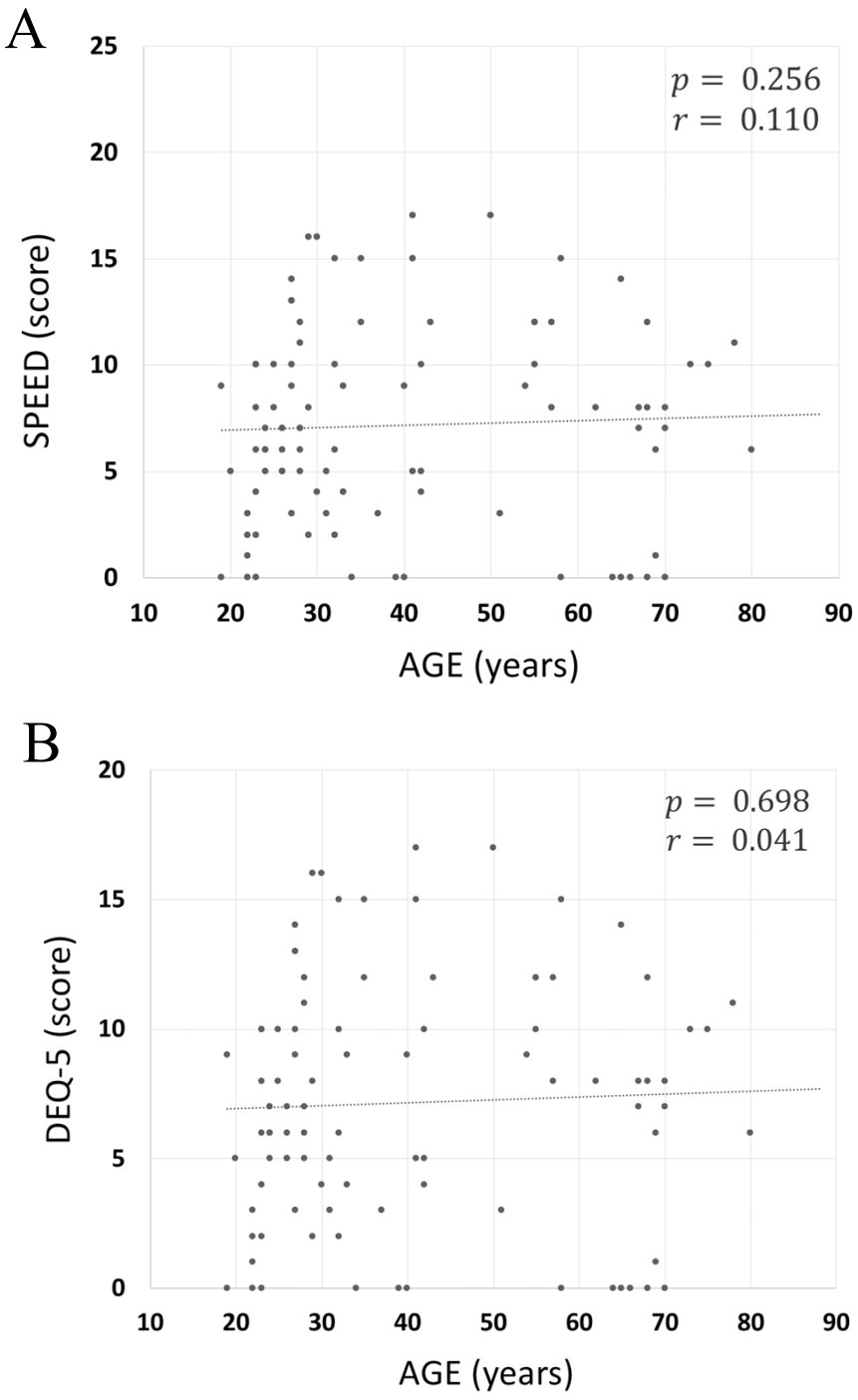
Figure 45. Correlations between Age and **A)** LWE from the UL and **B)** LWE from the LL assessed by slit lamp.
(r, Spearman correlation coefficient).

Concerning symptomatology, both OSDI and MQ questionnaires **(Figure 46A and 46B)** showed a weak correlation with age (fair; $r = 0.254, p = 0.01$ and $r = 0.241, p = 0.01$, respectively). On the other hand, no significant correlations were observed between SPEED, DEQ-5 (**Figure 47A and 47B**) and SANDE **(Figure 48)** with age (poor; $r = 0.110, p = 0.256$; $r = 0.041, p = 0.698$ and $r = 0.025, p = 0.798$, respectively).



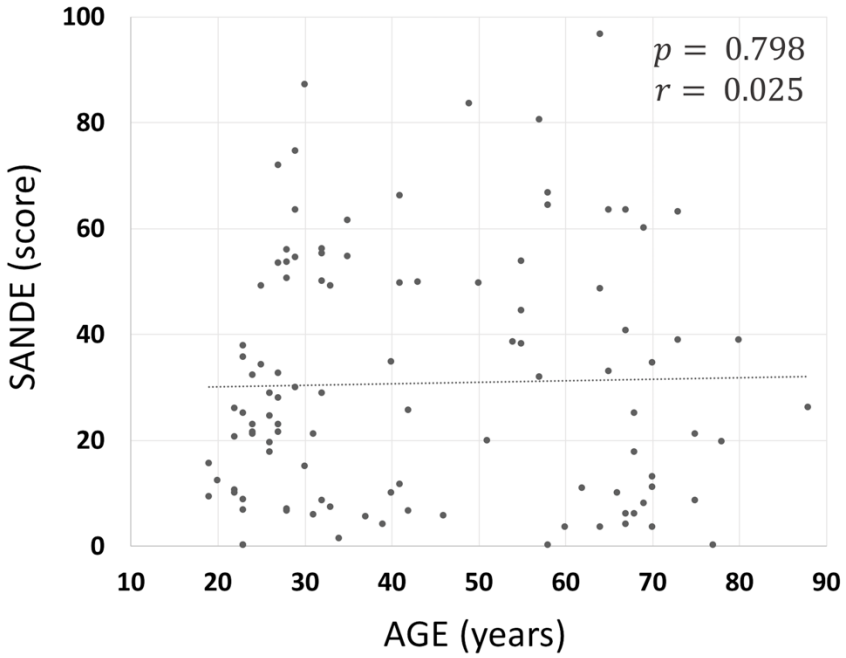
OSDI: The Ocular-surface-disease-index (scores)

Figure 46. Correlations between Age and **A)** OSDI questionnaire and **B)** McMonnies questionnaire.
(r, Pearson correlation coefficient).



Standard Patient Evaluation of Eye Dryness(scores); DEQ-5: Dry Eye Questionnaire (scores)

Figure 47. Correlations between Age and **A)** SPEED questionnaire **and B)** DEQ-5 questionnaire (r, Pearson correlation coefficient).



SANDE: Symptom Assessment in Dry Eye (scores).

Figure 48. Correlations between Age and SANDE questionnaire.
(r, Pearson correlation coefficient).

Demographic information, clinical parameters and symptomatology scores classified by age groups are shown in **Table 4**. The ocular surface differences between females and males was also analysed (see **Table 5**).

Table 4. Demographic information and comparison of the clinical parameters among age groups. Results expressed in mean±standard deviation *(in bold)* and median (IQR) for each parameter.

		Classified by AGE		
		A. <42	**B.** 42 to 65	**C.** >65
PARTICIPANTS (N)		61	24	24
AGE (years)		**28±6**	**55±8**	**72±5**
		27(8)	56(11)	70(8)
Female/Male		35/26	19/5	15/9
TFO	RE	**311±17**	**319±21**	**314±18**
		309(17)	315(20)	306(12)
	LE	**307±15**	**314±12**	**308±12**
		305(18)	309(23)	308(14)
	Inter-eye Diff	**-4±14**	**-6±16**	**-6±16**
		-1(13)	-5(16)	-1(16)
Keratograph K5M	TMHk	**0.24±0.06*†**	**0.28±0.06**	**0.29±0.10**
		0.23(0.08)	0.26(0.09)	0.28(0.12)
	BR Total	**0.80±0.31*†**	**1.37±0.67**	**1.50±0.37**
		0.23(0.08)	1.23(0.59)	1.52(0.38)
	LR	**0.40±0.23*†**	**0.80±0.40§**	**0.99±0.37**
		0.40(0.25)	0.75(0.43)	0.99(0.44)
	NIKBUT-first	**10.22±5.99**	**7.31±4.20**	**7.34±5.36**
		8.73(7.45)	6.31(3.20)	5.46(3.87)
	NIKBUT-avg	**13.14±5.42**	**10.47±4.55**	**10.86±5.80**
		13.51(7.61)	10.88(5.96)	9.77(8.13)
	MGL	**1.26±0.87*†**	**2.45±1.56**	**2.71±1.27**
		1.00(1.00)	2.00(1.25)	3.00(2.00)
Slit Lamp Assessment	Corneal Staining *(Oxford score)*	**0.61±0.76*†**	**1.46±0.97**	**1.25±1.03**
		0.00(1.00)	1.00(1.00)	1.00(1.00)
	Conjunctival Staining *(Oxford score)*	**1.07±0.89*†**	**1.83±0.70**	**2.54±0.88**
		1.00(2.00)	2.00(0.25)	2.00(1.00)
	TBUT	**5.11±2.71*†**	**3.52±1.03**	**3.30±1.43**
		4.13(2.49)	3.35(1.02)	2.84(1.42)

Lid Margin Assessment and MG grading	Lid margin thickness	**3.41±0.58** 3.00(1.00)	**4.17±0.76** 4.00(1.00)	**4.71±0.75** 5.00(0.00)
	Quality of the secretion expressed	**0.61±0.67*†** 1.00(1.00)	**1.26±0.69** 1.00(1.00)	**1.58±0.71** 2.00(1.00)
	Number of functional MGs	**0.21±0.41*†** 0.00(0.00)	**0.82±0.71** 1.00(1.00)	**1.21±0.72** 1.00(1.00)
	LWE UL	**0.83±0.74†** 1.00(1.00)	**1.13±0.54** 1.00(0.00)	**1.33±0.58** 1.00(1.00)
	LWE LL	**1.38±0.78†** 1.00(1.00)	**1.71±0.86** 2.00(1.00)	**2.04±0.88** 2.00(0.00)
Schirmer test		**14±9*†** 12(6)	**9± 3** 9(3)	**9±6** 8(6)
Symptomatology	**OSDI** (0-100)	**15.34±14.50†** 12.50(16.91)	**20.53±21.00** 12.50(23.96)	**25.31±16.91** 23.83(20.21)
	MQ (0-45)	**9.67±3.96** 10.00(5.00)	**11.46±6.93** 11.00(7.25)	**12.00±5.24** 12.00(7.75)
	DEQ-5 (0-20)	**7±5** 6(7)	**8±5** 10(8)	**6±4** 7(6)
	SPEED (0-28)	**7±5** 7(6)	**8±6** 8(10)	**8±4** 8(6)
	SANDE (0-100)	**29.93±21.72** 24.73(39.36)	**39.87±27.96** 38.52(43.22)	**23.55±19.88** 18.63(29.55)

TFO: Tear film osmolarity (mOsms/L); RE: Right Eye; LE: Left Eye; TMHk: Tear meniscus height (mm); Bulbar and limbal redness (BR and LR) were graded automatically by K5M software; NIKBUT-first: first rupture non-invasive Keratograph tear film break-up time (seconds); NIKBUT-avg: average of non-invasive Keratograph tear film break-up time (seconds); MGL: Meibomian gland loss graded with meiboscore scale; TBUT: Tear break-up time(seconds); LWE: Lid wiper ephiteliopathy (score). OSDI: The Ocular-surface-disease-index (scores); MQ: Mcmonnies (scores); DEQ-5: Dry Eye Questionnaire (scores); SPEED: Standard Patient Evaluation of Eye Dryness(scores) SANDE: Symptom Assessment in Dry Eye (scores).

*Indicates a statistically significant difference between groups A and B with p-value<0.05.

† Indicates a statistically significant difference between groups A and C with p-value<0.05.

§ Indicates a statistically significant difference between groups B and C with p-value<0.05.

Table 5. Comparison of the clinical parameters between females and males. Results expressed in mean±standard deviation *(in bold)* and median (IQR) for each parameter.

		Classified by SEX		
		Male	**Female**	
PARTICIPANTS (N)		40	70	**p-value**
AGE (years)		**41±19** 32(36)	**45±19** 41(37)	
TFO	RE	**313±16** 309(19)	**314±19** 312(15)	0.702
	LE	**307±13** 307(18)	**310±14** 306(18)	0.374
	Inter-eye Diff	**-5±16** 0(18)	**-4±15** -1(16)	0.729
Keratograph K5M	TMHk	**0.26±0.08** 0.25(0.07)	**0.25±0.08** 0.24(0.10)	0.219
	BR Total	**1.17±0.51** 1.07(0.70)	**1.03±0.54** 0.90(0.63)	0.160
	LR	**0.74±0.42** 0.53(0.53)	**0.70±0.40** 0.63(0.40)	0.604
	NIKBUT-first	**11.93±6.64** 10.94(9.96)	**7.21±4.07** 5.86(4.72)	0.0001*
	NIKBUT-avg	**14.99±5.66** 14.64(8.15)	**10.29±4.45** 9.56(6.54)	<0.0001*
	MGL	**1.75±1.17** 1.50(2.00)	**1.92±1.41** 2.00(1.00)	0.734
Slit Lamp Assessment	Corneal Staining *(Oxford score)*	**0.58±0.64** 0.50(1.00)	**1.17±1.05** 1.00(1.50)	0.002*
	Conjunctival Staining *(Oxford score)*	**1.28±1.04** 1.00(1.25)	**1.73±1.00** 2.00(1.00)	0.018*
	TBUT	**5.30±3.34** 3.88(2.99)	**3.81±1.30** 3.53(1.38)	0.010*

Lid Margin Assessment and MG grading	Lid margin thickness	**3.93±0.86** 4.00(2.00)	**3.81±0.86** 4.00(1.75)	0.504
	Quality of the secretion expressed	**1.08±0.83** 1.00(2.00)	**0.93±0.81** 1.00(1.00)	0.310
	Number of functional MG	**0.57±0.71** 0.00(1.00)	**0.58±0.72** 0.00(1.00)	0.986
	LWE UL	**0.92±0.79** 1.00(1.75)	**1.04±0.63** 2.00(1.00)	0.397
	LWE LL	**1.43±0.81** 1.00(1.00)	**1.71±0.78** 2.00(1.00)	0.085
Schirmer test		**13±9** 11(8)	**10.87±6.96** 10.00(6.75)	0.160
Symptomatology	**OSDI** (0-100)	**12.44±12.52** 8.33(12.45)	**22.34±18.15** 20.45(18.70)	0.001*
	MQ (0-45)	**9.10±4.03** 8.50(7.00)	**11.61±5.58** 11.50(6.75)	0.007*
	DEQ-5 (0-20)	**5±4** 5(6)	**8±4** 9(7)	0.0007*
	SPEED (0-28)	**6±4** 5(7)	**8±5** 8(7)	0.025*
	SANDE (0-100)	**22.27±19.25** 16.33(27.20)	**35.91±24.08** 32.35(36.66)	0.007*

TFO: Tear film osmolarity (mOsms/L); RE: Right Eye; LE: Left Eye; TMHk: Tear meniscus height (mm); Bulbar and limbal redness (BR and LR) were graded automatically by K5M software; NIKBUT-first: first rupture non-invasive Keratograph tear film break-up time (seconds); NIKBUT-avg: average of non-invasive Keratograph tear film break-up time (seconds); MGL: Meibomian gland loss graded with meiboscore scale; TBUT: Tear break-up time(seconds); LWE: Lid wiper ephiteliopathy in upper and lower eyelid(score). OSDI: The Ocular-surface-disease-index; MQ: Mcmonnies; DEQ-5: Dry Eye Questionnaire SPEED: Standard Patient Evaluation of Eye Dryness. SANDE: Symptom Assessment in Dry Eye.

***statistically significant differences between groups; p-value<0.05.**

Study II

Relationship between MGL assessed by NIM and the ocular surface parameters and symptomatology

The characteristics of the participants are summarized in **Table 6.** A total of 161 participants (mean age; 42 ± 17 years, 91 females and 70 males) were recruited for this study.

Table 6. Demographic data from the participants of the study.

N	161
AGE (years)	**42±17** 40(29)
AGE RANGE (years)	19 to 88
Male/ Female (%)	43/57
TFO	**309±17** 309(19)
TMHk	**0.26±0.08** 0.25(0.09)
LR	**0.70±0.37** 0.57(0.43)
BR	**1.11±0.49** 1.05(0.67)
NIKBUT- fr	**9.05±5.41** 7.01(6.81)
NIKBUT- avg	**12.02±5.26** 11.37(8.04)
TBUT	**4.36±2.07** 3.85(1.97)
SCHIRMER TEST	**11.83±7.14** 11.00(7.00)
OSDI	**17.47±15.65** 14.58(18.75)
SPEED	**7±5** 6(6)

TFO: tear film osmolarity(mOsms/L); TMHk: tear meniscus height (mm); Bulbar and limbal redness (BR and LR) were graded automatically by K5M software; NIKBUT-first: first rupture non-invasive Keratograph tear film break-up time (seconds); NIKBUT-avg: average of non-invasive Keratograph tear film break-up time (seconds); Schirmer test (mm); OSDI: The Ocular-surface-disease-index; SPEED: Standard Patient Evaluation of Eye Dryness.

3.2.1 Association and correlation between UL and LL

A contingency table was used to compare MGL between UL and LL (see **Table 7**) in order to know if it is necessary to evaluate the MGL in one eyelid or in both. Weighted kappa statistics showed no statistically significant agreement ($weighted\ k\ value = 0.2;\ p = 0.3;$ range $0.099 - 0.353,\ 95\%$ confidence limit) indicating no association between both eyelids. Despite it, a fair and statistically significant correlation was found between UL and LL (Spearman: $r = 0.3;\ p < 0.001$). For this reason, both eyelids should be assessed in order to know the overall condition of MG and its possible influence on the ocular surface state.

Table 7. Association between UL and LL according to MGL.

		Upper Eyelid (UL)				
	Total Meiboscore*	**0**	**1**	**2**	**3**	**Row Totals**
Lower Eyelid (LL)	**0**	21 44.68% 36.84%	22 46.81% 32.84%	4 8.51% 16.00%	0 0% 0%	*47*
	1	33 39.76% 57.89%	36 43.37% 53.73%	12 14.46% 48.00%	2 2.41% 28.51%	*83*
	2	3 13.04% 5.26%	8 34.78% 11.94%	9 39.13% 36.00%	3 13.04% 42.86%	*23*
	3	0 0% 0%	1 33.33% 1.49%	0 0% 0%	2 66.67% 28.57%	*3*
	Column Totals	*57*	*67*	*25*	*7*	156

*The *total meiboscore* is the sum of the meiboscore of both eyelids (0-6). Grade 0, no gland loss; grade 1, area of gland loss <33% of the total gland area; grade 2, area of gland loss 33%–67%; and grade 3, area of gland loss >67%

According to these findings, the participants of this study were divided by groups according to the total *meiboscore* (0-6), as proposed by Arita et al.[178]. Therefore, the group 1 was constituted by participants who presented a total meiboscore of 0; group 2 by participants who showed a total meiboscore of 1; group 3 by participants who

showed a total meiboscore of 2; group 4 by participants who showed a total meiboscore of 3 and group 5 by participants who showed a total meiboscore of 4,5 or 6. According to the groups established groups, the percentage range of MGL was 0, 0-16.6, 16.5-33,33-49.5 and >50, for groups 1, 2, 3, 4 and 5 respectively. **Table 8** shows the distribution of the sample as a function of the total meiboscore, and its demographic characteristics**.**

Table 8. Characteristics and distribution of the sample according the MGL grade (total meiboscore). Results expressed in mean±standard deviation *(in bold)* and median (IQR) for each parameter.

Total Meiboscore*	Group 1	Group 2	Group 3	Group 4	Group 5
	0 (reference)	**1**	**2**	**3**	**4+5+6**
MGL (%)	0	0-16.5	16.5-33	33-49.5	>50
N (%)	21 (13.04%)	59 (36.65%)	44 (27.33%)	20 (12.42%)	17 (10.55%)
AGE (years)	**34±11** 32(18)	**37±15** 32(19)	**46±17** 44(25)	**50±18** 53(34)	**60±18** 64(18)
Female (%)	9/91 9.9%	33/91 36.3%	27/91 29.7%	8/91 8.8%	12/91 13.2%
Male (%)	12/70 17.1%	25/70 35.7%	17/70 24.3%	12/70 17.1%	4/70 5.7%

*The *total meiboscore* is the sum of the meiboscore of both eyelids (0-6).
MGL: Meibomian gland loss

The results from the tables of the next sections are also presented in a boxplot graphics in order to visualize the trends appearing in the data obtained.

3.2.2 Symptomatology assessment.

Results regarding subjective symptomatology is showed in **Figure 49** and **Table 9**. No statistically significant differences were found in OSDI ($p = 0.385$) and SPEED ($p = 0.506$) questionnaires among different MGL groups.

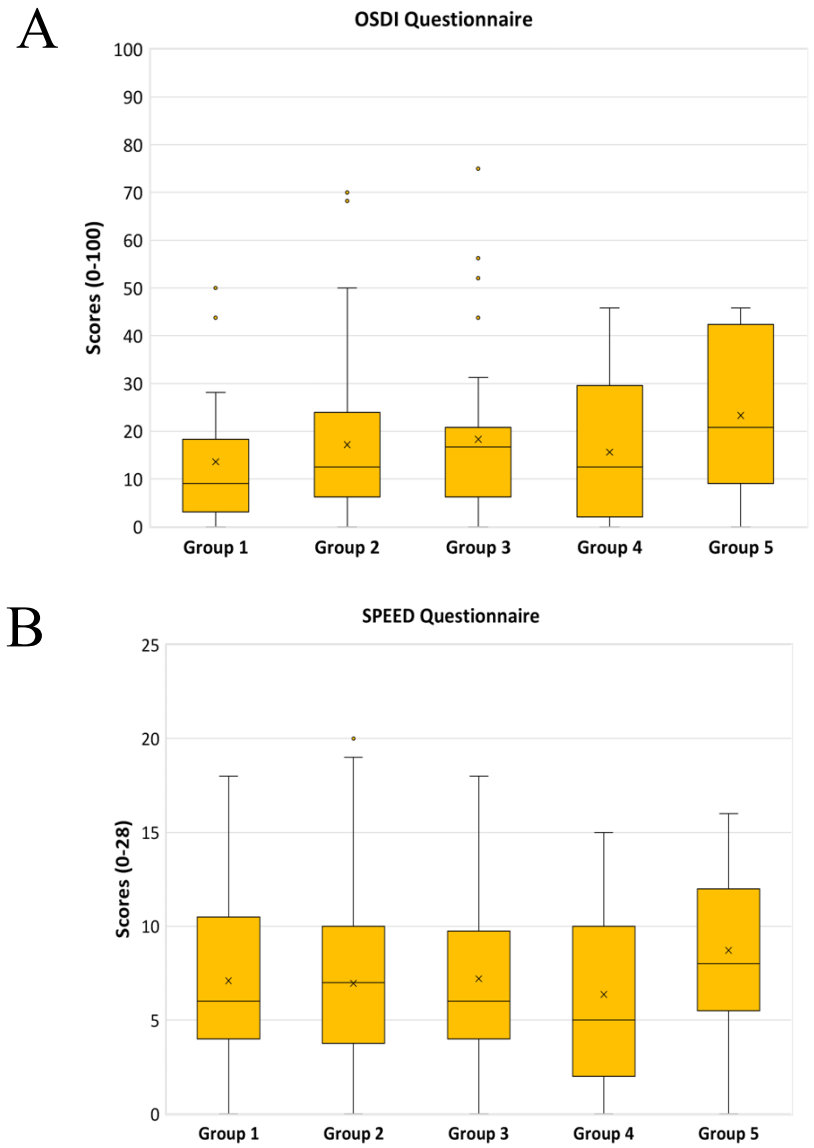


Figure 49. Symptomatology boxplots graphs from **(A)** OSDI questionnaire **(B)** SPEED Questionnaire

MEIBOSCORE GROUP (MGL%)	SYMPTOMATOLOGY	
	OSDI (0-100)	**SPEED (0-28)**
Group 1 (0)	**13.60±13.75** 9.09(12.50)	**7±5** 6(6)
Group 2 (0-16.5)	**17.18±15.37** 12.50 (16.66)	**7±5** 7(6)
Group 3 (16.5-33)	**18.30±16.76** 16.60 (13.54)	**7±5** 6(5)
Group 4 (33-49.5)	**15.61±15.21** 12.50 (23.05)	**6±5** 5 (8)
Group 5 (>50)	**23.28±16.27** 20.83 (27.27)	**9±4** 8(6)
p-value	*0.385*	*0.506*

OSDI: The Ocular-surface-disease-index (scores); SPEED: Standard Patient Evaluation of Eye Dryness (scores)

***statistically significant differences among groups; p<0.05**.

Table 9. Comparison of symptomatology scores among different MGL grades. Results expressed in mean±standard deviation *(in bold)* and median (IQR) for each

3.2.3 Classical Clinical Parameters.

Classical clinical parameters result for each group are shown in **Figures 50 and 51** as well as **in Table 10.** Statistically significant differences were found among groups in TFO ($p = 0.023$), corneal ($p = 0.015$) and conjunctival staining ($p = 0.004$). For TFO, there were statistically significant differences between group 5 and groups 1,2 and 3 ($p = 0.029$; $p = 0.014$; $p = 0.001$, respectively). No differences were found among groups 1, 2 and 3 (p=0.358); neither on between groups 4 and 5 ($p = 0.210$). For the corneal staining, there were statistically significant differences between group 5 and groups 1 and 2 ($p = 0.045$ and 0.003, respectively). No differences were found among groups 3, 4 and 5 ($p = 0.191$). Regarding conjunctival staining, there were also statistically significant differences between group 5 and groups 1 and 2 ($p = 0.027$ and 0.007, respectively) and between group 4 and groups 1 and 2 ($p = 0.045$ and 0.014,

respectively). No differences were found among groups 1 and 2 (p=0.953) neither among groups 3, 4 and 5 ($p = 0.303$). On the other hand, no statistically significant differences were found among the five groups for TBUT ($p = 0.249$) and Schirmer test ($p = 0.160$).

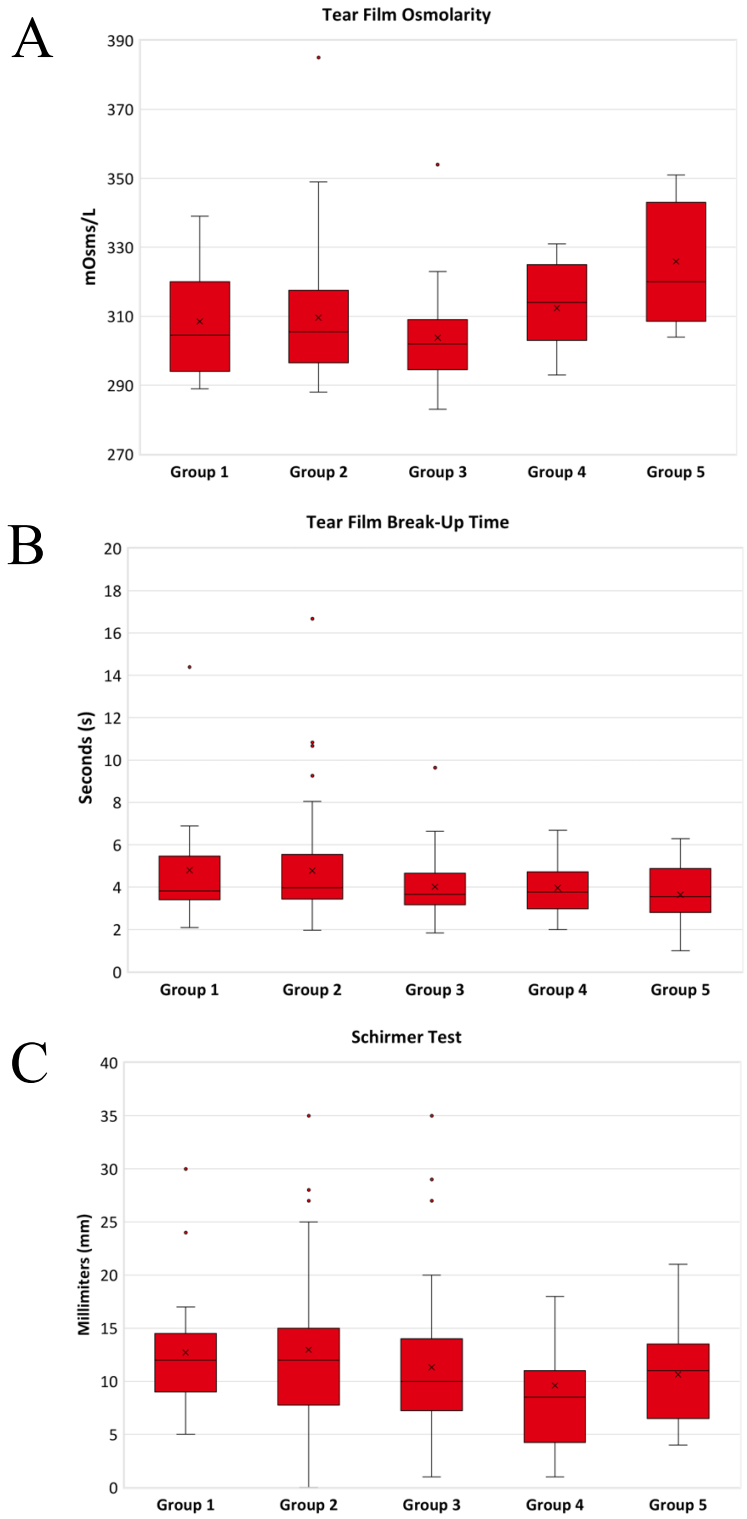


Figure 50. Classical clinical parameters boxplots graphs from **(A)** Tear Film Osmolarity **(B)** Tear film break-up time and **(C)** Schirmer test.

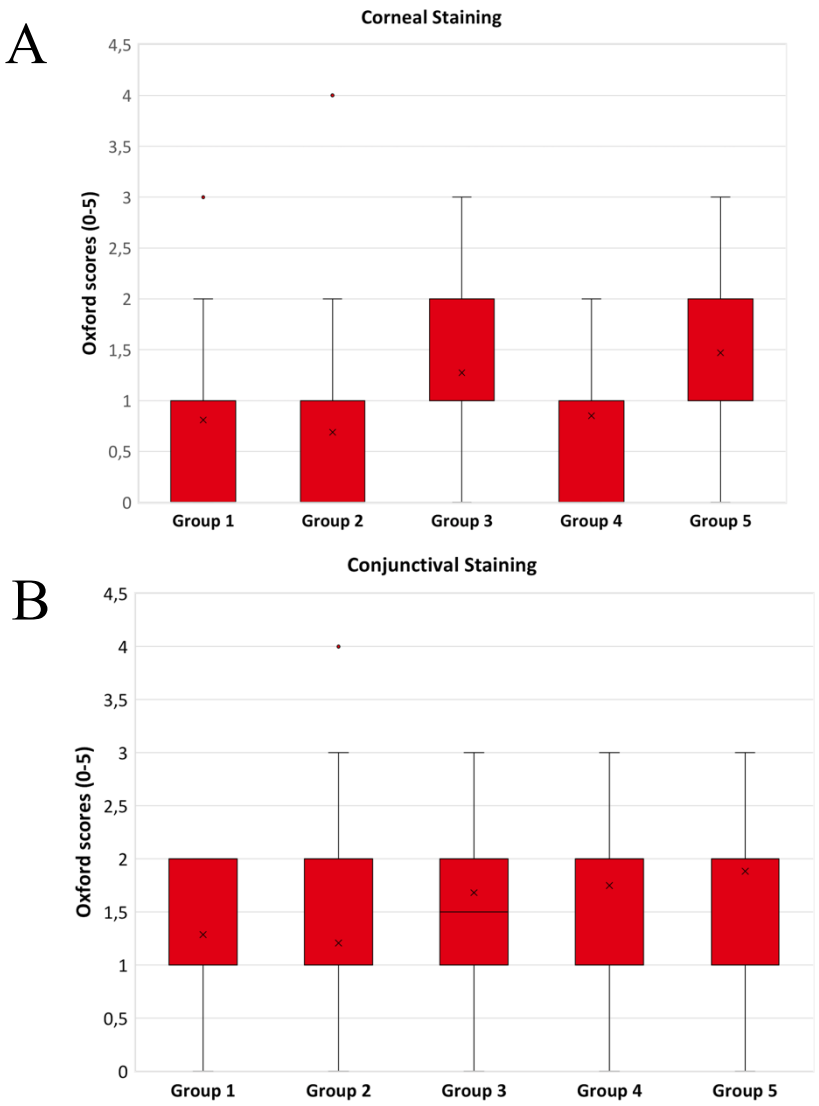


Figure 51. Classical clinical parameters boxplots graphs from **(A)** Corneal staining and **(B)** Conjunctival staining.

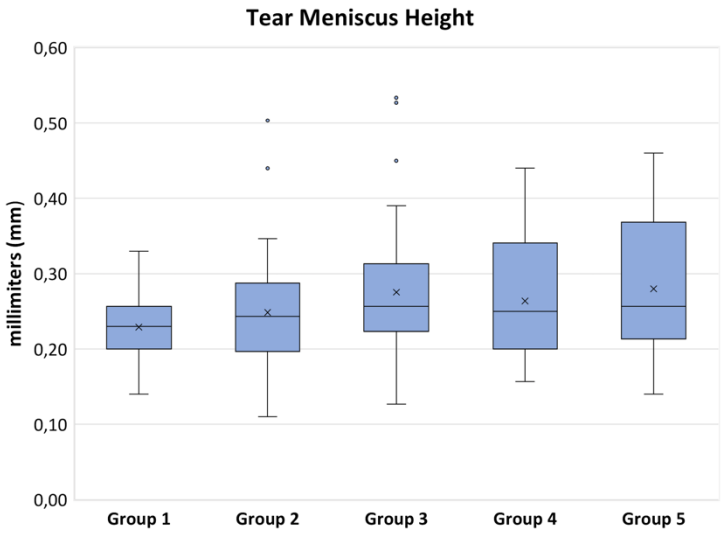
Table 10. Comparison of classical clinical parameters among different MGL groups. Results expressed in mean±standard deviation *(in bold)* and median (IQR) for each parameter.

MEIBOSCORE GROUP (MGL%)	CLASSICAL CLINICAL PARAMETERS				
	TFO	**TBUT**	**SCHIRMER**	**CORNEAL STAINING**	**CONJUNCTIVAL STAINING**
Group 1 (0)	**308±16** 305(21)	**4.86±2.52** 3.81(2.02)	**13±6** 12(4)	**0.76±0.70** 1.00 (1.00)	**1.19±0.68** 1.00 (1.00)
Group 2 (0-16.5)	**310±19** 306 (20)	**4.85±2.57** 3.97(1.96)	**13±8** 12(7)	**0.62±0.85** 0.00(1.00)	**1.20±0.83** 1.00 (1.00)
Group 3 (16.5-33)	**304±15** 302(14)	**4.04±1.46** 3.73(1.47)	**11±7** 10(6)	**0.98±0.86** 1.00 (1.00)	**1.67±1.02** 1.50 (1.00)
Group 4 (33-49.5)	**312±12** 314(14)	**3.96±1.22** 3.77(1.67)	**10±7** 9(6)	**0.85±0.67** 1.00 (1.00)	**1.75±0.79** 2.00 (1.00
Group 5 (>50)	**326±18** 320(30)	**3.64±1.51** 3.54(1.53)	**11±4** 11(6)	**1.47±1.23** 1.00 (1.00)	**1.88±0.93** 2.00 (1.00)
p-value	*0.023**	*0.249*	*0.160*	*0.015**	*0.004**

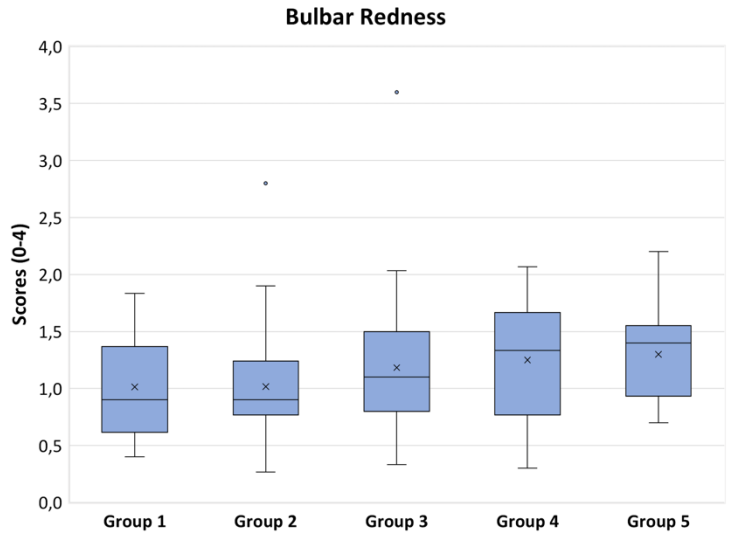
TFO: tear film osmolarity (mOsms/L); TBUT: tear break-up time (seconds); Schirmer test (mm); Corneal and conjunctival staining were graded using Oxford Staining Score System.
*** statistically significant differences among groups; p<0.05.**

3.2.4 Keratograph 5M Automated Measurements

Figures 52 and 53 as well as **Table 11** show the automated measurements obtained by K5M for each group**.** Statistically significant differences in BR were found between groups 5 and 1 $(p = 0.043)$ and groups 5 and 2 $(p = 0.010)$. On the contrary, no statistically significant differences were found among groups for TMHk $(p = 0.405)$, LR $(p = 0.063)$, NIKBUT-first $(p = 0.213)$ and NIKBUT-avg $(p = 0.427)$. Furthermore, no statistically significant differences were found among MGL groups and LLP (see **Table 12**).



A



B

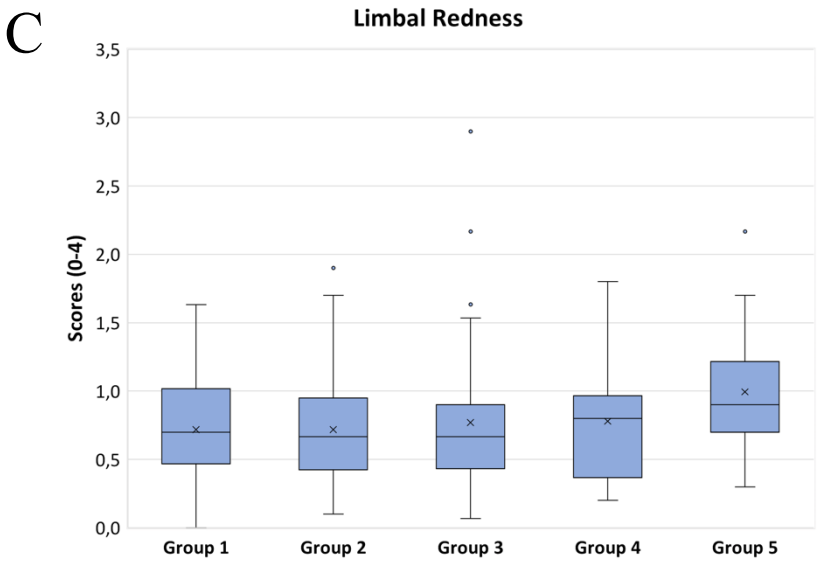


Figure 52. K5M automated measurements (A) Tear Meniscus Height (B) Bulbar Redness (C) Limbal Redness.

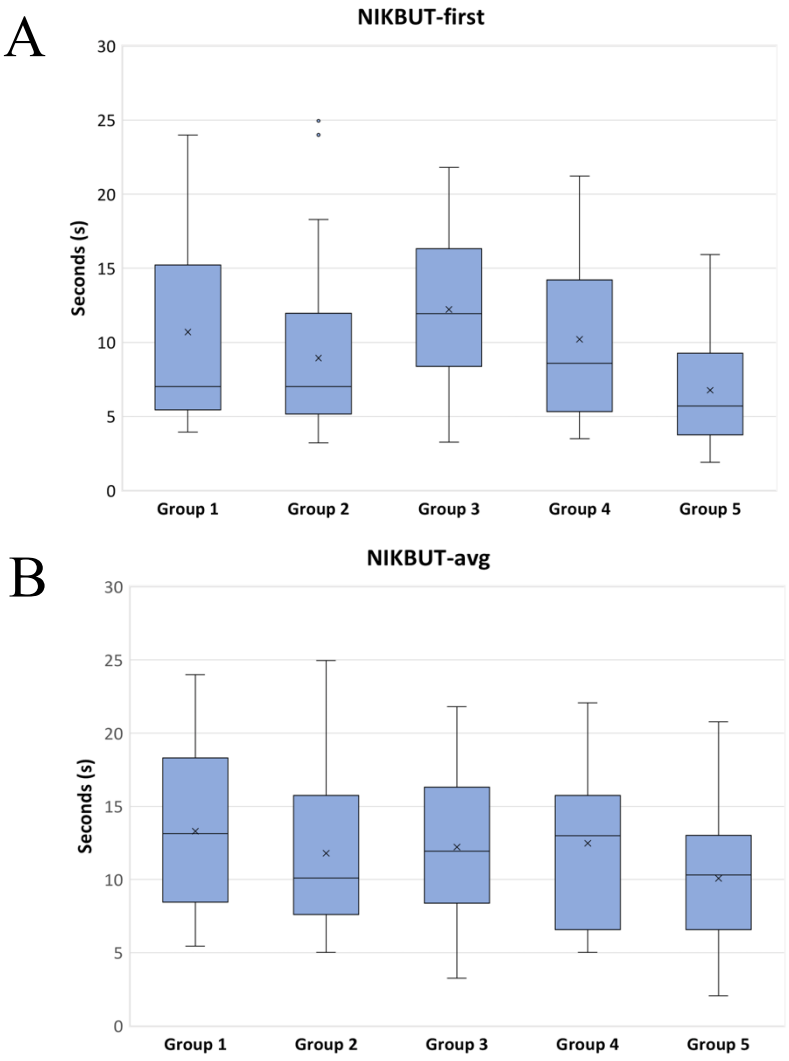


Figure 53. K5M automated measurements **(A)** NIKBUT-first**(B)** NIKBUT-avg.

Table 11. Comparison of K5M automated measurements among different MGL groups. Results expressed in mean±standard deviation *(in bold)* and median (IQR) for each parameter.

MEIBOSCORE GROUP (MGL%)	AUTOMATED MEASUREMENTS BY K5M				
	TMHk	**BR**	**LR**	**NIKBUT-first**	**NIKBUT-avg**
Group 1 (0)	**0.23±0.05** 0.23(0.04)	**1.00±0.46** 0.90(0.53)	**0.68±0.35** 0.47(0.40)	**11.23±6.82** 7.77(7.84)	**13.81±6.15** 13.42 (7.42)
Group 2 (0-16.5)	**0.26±0.07** 0.24(0.09)	**0.99±0.44** 0.90 (0.45)	**0.62±0.32** 0.58(0.36)	**8.79±5.12** 7.01(6.61)	**11.80±5.02** 10.26 (8.05)
Group 3 (16.5-33)	**0.27±0.09** 0.26(0.08)	**1.16±0.55** 1.07(0.67)	**0.70±0.40** 0.63(0.53)	**8.83±4.95** 8.11(6.92)	**12.32±4.97** 11.92(7.72)
Group 4 (33-49.5)	**0.26±0.80** 0.25(0.11)	**1.25±0.53** 1.33(0.78)	**0.76±0.37** 0.70 (0.72)	**10.19±5.96** 8.59(7.36)	**12.48±5.52** 12.98 (9.15)
Group 5 (>50)	**0.28±0.10** 0.26(0.15)	**1.30±0.41** 1.40(0.57)	**0.92±0.41** 0.85(0.37)	**6.76±4.08** 5.59(4.96)	**10.07±4.95** 10.30(5.30)
p-value	*0.405*	*0.040**	*0.063*	*0.213*	*0.427*

TMHk: tear meniscus height (mm); Bulbar (BR) and limbal redness (LR) were graded automatically by K5M software; NIKBUT-first: first rupture non-invasive Keratograph tear film break-up time (seconds); NIKBUT-avg: average of non-invasive Keratograph tear film break-up time (seconds).

***statistically significant differences among groups; p<0.05.**

Table 12. Comparison of lipid layer patterns among different MGL groups. Results expressed in n (%).

MEIBOSCORE GROUP (MGL%)						
LIPID LAYER PATTERNS	**Group 1** (0)	**Group 2** (0-16.5)	**Group 3** (16.5-33)	**Group 4** (33-49.5)	**Group 5** (>50)	**Total**
0	1(4.8)	2(3.4)	2(4.5)	0(0)	1(5.9)	6 (3.7)
1	5(23.8)	6(10.2)	7(15.9)	3(15)	1(5.9)	22(13.7)
2	4(19)	9(15.3)	5(11.4)	6(30)	3(7.6)	27(16.8)
3	8(38.1)	31(52.2)	19(43.2)	9(45.0)	8(47.1)	75(46.6)
4	3(14.3)	11(18.6)	11(25)	2(10)	4(23.5)	31(19.3)
Total	21	59	44	20	17	161
p-value	0.858					

ipid Layer Patterns (Guillon): 0= None or not visible; 1= Meshwork (open/tight); 2= Wave (Meshwork and Wave); 3= Amorphous (Wave and Amorphous) and 4= Colours (Wave and Colours/ Amorphous and Colours and Colours).

***statistically significant differences among groups; p<0.05.**

As aforementioned, the age is a relevant factor in MG morphology and it has been demonstrated in the **Study I**, it is also relevant on the ocular surface. Therefore, **Table 13** shows the relationship between the MGL and several ocular surface parameters. It is also included other analysis of the relationship taking the age into account age as a covariant.

Table 13. Correlation coefficients of MGL and ocular surface parameter and same coefficient correlations applying age as a covariant.

	MGL (Covariant: **Age)**	
	Correlation coefficient	*p*
DED Questionnaires		
OSDI	0.011	0.914
SPEED	0.012	0.904
K5M Parameters		
TMHk	0.059	0.563
BR	-0.003	0.969
LR	-0.044	0.668
NIKBUT-fr	-0.095	0.345
NIKBUT-avg	-0.054	0.597
Lipid Layer Patterns	-0.041	0.611
Classical Clinical Parameters		
TFO	0.088	0.393
Corneal staining	0.208	0.041*
Conjunctival staining	-0.004	0.966
TBUT	-0.163	0.112
Schirmer test	-0.065	0.527
MG Features		
MG quality secretion	0.044	0.587
Functional MG	0.145	0.074

TFO: Tear film osmolarity (mOsms/L); TMHk: Tear meniscus height (mm); Bulbar and limbal redness (BR and LR) were graded automatically by K5M software (scores); NIKBUT-first: first rupture non-invasive Keratograph tear film break-up time (seconds); NIKBUT-avg: average of non-invasive Keratograph tear film break-up time (seconds); MGL: Meibomian gland loss graded with meiboscore scale; LLP: Lipid layer patterns; TBUT: Tear break-up time(seconds); OSDI: The Ocular-surface-disease-index (scores); SPEED: Standard Patient Evaluation of Eye Dryness (scores).

(r, Pearson and Spearman correlations)
*statistically significant; p-value<0.05.

Study III

Relationship between new objective MG morphological parameters and the ocular surface parameters

The results of this study are divided into two sections:

- 1º section: It shows the relationship between objective MG morphological parameters and the most relevant ocular surface clinical parameters.
- 2º section: It shows the relationship between objective MG irregularity and the most relevant ocular surface clinical parameters in a group of participants that showed a MGL <32%.

3.3.1 Relationship between objective MG morphological parameters and the most relevant ocular surface clinical parameters.

The automated algorithm processed successfully 149 (out of 161) meibography images from the UL. **Table 14, 15 and 16** show the demographic data (general information, MG features and clinical parameters, respectively) of the sample evaluated.

Table 14. General information of the sample evaluated.

N	149
AGE (years)	42± 17 40 (28)
SEX (Female /Male)	80/69

Table 15. MG morphological features of the sample evaluated.

MG Morphological Features						
	Automated and Objective Parameters				Slit-lamp Assessment	
	Objective MGL (%)	**Mean Width** (mm)	**Mean Length** (mm)	**Number of glands**	**Quality of secretion** (score)	**Functional MG** (score)
Mean±SD	20.32 ± 13.95	0.39 ± 0.07	2.55 ± 0.60	24 ± 7	0.97± 0.81	0.51± 0.70
Median(IQR)	17.50(17.00)	0.39(0.08)	2.57 (0.84)	24(8)	1 (2)	0 (1)

MG: Meibomian glands; MGL: Meibomian gland loss; SD: Standard deviation; IQR: Interquartile rang

Table 16. Clinical parameters of the sample evaluated.

Ocular Surface Parameters											
	OSDI (score)	**SPEED** (score)	**TFO** (mOsms/L)	**BR** (score)	**TMHk** (mm)	**NIBUT-AVG** (seconds)	**TBUT** (seconds)	**SCHIRMER** (mm)	**LWE UL** (score)	**Corneal Staining** (score)	**Conjunctival Staining** (score)
Mean±SD	16.84±14.96	7± 5	310±18	1.10±0.45	0.26±0.08	12.00±5.18	4.44±2.11	12± 7	1.01±0.66	0.8 ±0.9	1.47± 0.90
Median (IQR)	14.11(16.42)	6(6)	306 (21)	1.03 (0.63)	0.25 (0.09)	11.37 (7.97)	3.94 (1.93)	11 (7)	1 (0)	1(1)	1 (1)

SD: Standard deviation; IQR: Interquartile range; OSDI: The Ocular-surface-disease-index; SPEED: Standard Patient Evaluation of Eye Dryness ; TFO: Tear film osmolarity; BR: Bulbar redness was graded automatically by K5M software; TMHk: Tear meniscus height; NIKBUT-avg: average of non-invasive Keratograph tear film break-up time; TBUT: Tear break-up time; LWE UL: Lid wiper epitheliopathy severity of the upper eyelid.

In order to know the relationship between the new MG morphology parameters and the most relevant ocular surface parameters, the correlation coefficients were obtained (see **Table 17**). As well, the correlation coefficients of those MG morphology parameters which demonstrated to be significantly influenced by age were obtained where age was included as a covariant (see **Table 18**).

Table 17. Correlation coefficients of objective MG morphological parameters and ocular surface parameters.

	Objective MGL		Mean Length		Mean Width		Number of Glands	
	Correlation coefficient	*p*	**Correlation coefficient**	*p*	**Correlation coefficient**	*p*	**Correlation coefficient**	*p*
AGE	0.253	0.002*	-0.214	0.009*	-0.196	0.017*	-0.114	0.168
SEX	-0.034	0.680	0.158	0.056	-0.068	0.414	-0.062	0.454
DED Questionnaires								
OSDI	0.062	0.451	0.012	0.880	-0.092	0.266	0.029	0.720
SPEED	0.006	0.934	0.037	0.653	-0.030	0.717	-0.034	0.681
Tear Film Osmolarity								
TFO	-0.078	0.459	0.014	0.890	0.039	0.705	-0.094	0.369
Keratograph 5M								
TMHk	0.274	0.0008*	-0.172	0.037*	-0.038	0.642	-0.029	0.722
BR	0.177	0.032*	-0.214	0.0095*	-0.119	0.152	-0.140	0.092
NIKBUT-avg	0.076	0.360	-0.042	0.613	0.097	0.239	-0.147	0.075
ClassicalClinical Parameters								
Corneal Staining	0.049	0.554	-0.009	0.907	-0.080	0.332	-0.010	0.894
Conjunctival Staining	0.133	0.105	-0.139	0.091	-0.130	0.113	-0.071	0.388
LWE UL	0.000	0.991	-0.049	0.551	-0.017	0.830	-0.084	0.312
TBUT	-0.110	0.180	0.156	0.057	0.210	0.010*	0.082	0.319
Schirmer test	-0.027	0.741	0.038	0.644	-0.018	0.820	-0.027	0.735
MG Features								
MG quality secretion	0.100	0.226	-0.125	0.129	-0.048	0.562	-0.127	0.124
Functional MG	0.106	0.199	-0.056	0.500	-0.001	0.562	-0.071	0.392

DED: Dry eye disease; OSDI: The Ocular-surface-disease-index (score); SPEED: Standard Patient Evaluation of Eye Dryness (score) ;TFO: Tear film osmolarity (mOsms/L); TMHk: Tear meniscus height (mm); BR: Bulbar redness was graded automatically by K5M software; NIKBUT-avg: average of non-invasive Keratograph tear film break-up time (seconds); LWE UL: Lid wiper epitheliopathy severity of the upper eyelid (severity score); TBUT: Tear break-up time(seconds); MG: Meibomian glands.
(r, Pearson and Spearman)
***statistically significant; p<0.05.**

Table 18. Correlation coefficients of objective MG morphological parameters and ocular surface parameters applying age as a covariant.

	Objective MGL (Covariant: **AGE**)		**Mean Length** (Covariant: **AGE**)		**Mean Width** (Covariant: **AGE**)	
	Correlation coefficient	*p*	**Correlation coefficient**	*p*	**Correlation coefficient**	*p*
DED Questionnaires						
OSDI	0.017	0.878	0.058	0.605	-0.147	0.185
SPEED	-0.047	0.671	0.047	0.676	-0.103	0.353
Tear Film Osmolarity						
TFO	0.005	0.967	-0.006	0.956	0.028	0.800
Keratograph 5M						
TMHk	0.119	0.285	-0.030	0.786	-0.086	0.440
BR	-0.142	0.199	0.121	0.313	0.159	0.152
NIKBUT-avg	-0.032	0.771	0.075	0.500	-0.045	0.687
Classical Clinical Parameters						
Corneal Staining	-0.050	0.654	0.153	0.169	0.054	0.630
Conjunctival Staining	-0.178	0.108	0.119	0.286	0.080	0.470
LWE UL	-0.047	0.672	0.060	0.588	0.067	0.560
TBUT	-0.081	0.468	-0.008	0.945	0.210	0.057
Schirmer test	-0.050	0.655	0.046	0.679	0.013	0.909
MG Features						
MG quality secretion	-0.098	0.380	0.136	0.219	0.063	0.569
Functional MG	0.144	0.195	-0.058	0.605	-0.013	0.910

DED: Dry eye disease; OSDI: The Ocular-surface-disease-index (score); SPEED: Standard Patient Evaluation of Eye Dryness (score) ;TFO: Tear film osmolarity (mOsms/L); TMHk: Tear meniscus height (mm); BR: Bulbar redness was graded automatically by K5M software; NIKBUT-avg: average of non-invasive Keratograph tear film break-up time (seconds); LWE UL: Lid wiper epitheliopathy severity of the upper eyelid (severity score); TBUT: Tear break-up time(seconds); MG: Meibomian glands

(r, Spearman correlation)

***statistically significant; p<0.05.**

3.3.2 Relationship between objective MG irregularity and the most relevant ocular surface clinical parameters.

The objective MG irregularity was assessed in a group of participants with a MGL $< 32\%$ since it is a morphological feature only observed when the MG are still present. A total of 122 participants (out of 161) showed a MGL $< 32\%$. **Table 19, 20 and 21** show the demographic data (general information, MG features and clinical parameters, respectively) of the sample evaluated.

Table 19. General information of the sample evaluated. Results expressed in mean±SD and median and IQR.

	< 32% Objective MGL
N	122
AGE (years)	40 ± 17 36 (25)
SEX (Female/Male)	64/58

MGL: Meibomian gland losss

Table 20. MG morphological features of the sample evaluated. Results expressed in mean±SD and median and IQR.

MG Morphological Features						
	Automated and Objective Parameters					**Slit-Lamp Assessment**
	Irregularity	**Objective MGL** (%)	**Mean Width** (mm)	**Mean Length** (mm)	**Number of glands**	**Quality of secretion** (score)
Mean±SD **Median(IQR)**	22.11 ± 40.56 5.30(29.16)	15.39 ± 8.44 15.00(13.75)	0.41 ± 0.06 0.40(0.08)	2.71 ± 0.52 2.68 (0.59)	26 ± 6 25(7)	2.71 ± 0.52 2.68 (0.59)

MG: Meibomian glands; MGL: Meibomian gland loss; SD: Standard deviation; IQR: Interquartile range

Table 21. Clinical parameters of the sample evaluated. Results expressed in mean±SD and median and IQR.

Ocular Surface Parameters									
	OSDI (score)	**TFO** (mOsms/L)	**BR** (score)	**TMHk** (mm)	**NIBUT-AVG** (seconds)	**TBUT** (seconds)	**SCHIRMER** (mm)	**LWE UL** (score)	**Corneal Staining** (score)
Mean±SD	16.31±14.62	309.8 ± 17.8	1.06 ± 0.45	0.25 ± 0.07	12.02 ± 0.52	4.56 ± 2.23	12 ± 8	1.0 ± 0.7	0.8 ± 0.9
Median (IQR)	12.50(16.26)	306.0 (21.5)	0.97(0.53)	0.24(0.08)	2.68 (0.59)	3.97 (1.90)	11(7)	1(0)	1(1)

SD: Standard deviation; IQR: Interquartile range; OSDI: The Ocular-surface-disease-index; TFO: Tear film osmolarity; BR: Bulbar redness was graded automatically by K5M software; TMHk: Tear meniscus height; NIKBUT-avg: average of non-invasive Keratograph tear film break-up time; TBUT: Tear break-up time; LWE UL: Lid wiper epitheliopathy severity of the upper eyeli

Table 22. Correlation coefficients of objective MG irregularity and MG morphological features and ocular surface parameters.

Objective MG Irregularity		
	Correlation Coefficient	**p**
AGE	-0.117	0.198
SEX	0.010	0.916
MG Morphological Features		
Objective MGL	-0.073	0.421
Mean Width	0.516	<0.0001*
Mean Length	0.211	0.020*
Number of glands	0.009	0.922
Quality of secretion	-0.026	0.778
Ocular Surface Parameters		
OSDI	0.015	0.874
TFO	-0.064	0.578
BR	-0.045	0.626
TMHk	0.008	0.928
NIBUT AVG	0.068	0.458
TBUT	0.108	0.234
SCHIRMER	0.090	0.322
LWE UL	-0.078	0.398
Corneal Staining	-0.071	0.436

OSDI: The Ocular-surface-disease-index (score);TFO: Tear film osmolarity (mOsms/L); BR: Bulbar redness was graded automatically by K5M software; TMHk: Tear meniscus height (mm); NIKBUT-avg: average of non-invasive Keratograph tear film break-up time (seconds); LWE UL: Lid wiper epitheliopathy severity of the upper eyelid (severity score); TBUT: Tear break-up time(seconds); MG: Meibomian glands; MGL: Meibomian gland loss.
(r, Spearman correlation)
***statistically significant; p- value < 0.05**

In the light of these results, the participants were classified according to three different levels of MG irregularity as follow: *low, medium and high*. These groups who showed different irregularity were compared (see **Table 23**) and afterward, the relationship between each level of MG irregularity and the ocular surface parameters were obtained (see **Table 24**).

Table 23. Demographic data from the three different levels of MG irregularity and its comparison. Results expressed in mean±SD and median and IQR.

		LOW (G1)	MEDIUM (G2)	HIGH (G3)	
N		78	22	22	**p**
AGE (years)	Mean±SD Median(IQR)	42 ± 18 36(28)	37 ± 12 34(14)	38 ± 16 41(22)	0.580
SEX Female/Males		40/30	12/10	12/10	0.942
MG Morphological Features					
Objective MGL (%)	Mean±SD Median(IQR)	15.79 ± 8.40 14.50(12.75)	14.00± 8.45 11.50(13.75)	15.36 ± 8.82 16.50(14.50)	0.674
Mean Width (mm)	Mean±SD Median(IQR)	0.39 ± 0.05 0.38(0.07)	0.43± 0.06 0.43(0.09)	0.46± 0.06 0.44(0.07)	<0.001*
Mean Length (mm)	Mean±SD Median(IQR)	2.65 ± 0.51 2.65(0.55)	2.70± 0.40 2.69(0.43)	2.91 ± 0.60 3.03(0.97)	0.117
Number of Glands	Mean±SD Median(IQR)	26 ± 6 25(7)	27± 7 27(9)	24 ± 6 24(10)	0.353
Quality of Secretion (score)	Mean±SD Median(IQR)	0.51 ± 0.73 0(1)	0.41 ± 0.59 0(1)	0.32 ± 0.57 0(1)	0.511
Ocular Surface Parameters					
OSDI (score)	Mean±SD Median(IQR)	17.14 ± 14.58 14.58(16.47)	18.41 ± 18.47 11.46(18.23)	11.35 ± 8.89 9.37(15.34)	0.307
TFO (mOsms/L)	Mean±SD Median(IQR)	308 ± 15 306(21)	320 ± 30 309(31)	307 ± 15 306(22)	0.666
BR (score)	Mean±SD Median(IQR)	1.09 ± 0.47 1.00(0.64)	0.91 ± 0.39 0.87(0.50)	1.09± 0.41 1.03(0.67)	0.301
TMHk (mm)	Mean±SD Median(IQR)	0.25 ± 0.08 0.24(0.09)	0.25 ± 0.06 0.24(0.10)	0.25 ± 0.06 0.25(0.08)	0.894
NIKBUT AVG (seconds)	Mean±SD Median(IQR)	11.35 ± 5.19 10.13(6.79)	13.31 ± 4.97 15.11(7.71)	13.12 ± 5.46 13(7.46)	0.125
TBUT (seconds)	Mean±SD Median(IQR)	4.27 ± 2.00 3.90(1.55)	5.44 ± 2.20 5.11(3.05)	4.71 ± 2.83 4.03(1.46)	0.037*
SCHIRMER (mm)	Mean±SD Median(IQR)	11.87 ± 6.64 11(6.50)	13.68 ± 10.39 10.50(8.50)	13.68 ± 7.74 11(7.50)	0.666
LWE UL (score)	Mean±SD Median(IQR)	0.97 ± 0.65 1(0)	0.91 ± 0.75 1(1)	1.05 ± 0.72 1(1.25)	0.687
Corneal Staining (score)	Mean±SD Median(IQR)	0.81 ± 0.87 1(1)	0.73 ± 0.98 0.50(1)	0.68 ± 0.72 1(1)	0.666

SD: Standard deviation; IQR: Interquartile range; OSDI: The Ocular-surface-disease-index; TFO: Tear film osmolarity; BR: Bulbar redness was graded automatically by K5M software; TMHk: Tear meniscus height; NIKBUT-avg: average of non-invasive Keratograph tear film break-up time; TBUT: Tear break-up time; LWE UL: Lid wiper epitheliopathy severity of the upper eyelid.

***statistically significant among groups; p- value < 0.05**

Statistically significant differences were found among groups in mean width ($p < 0.001$) and TBUT ($p = 0.037$). Regarding mean width, there were statistically significant differences between group 1 and groups 2 and 3 ($p = 0.01$ and $p = 0.006$) but no differences were found between groups 2 and 3 ($p = 0.663$). In the case of TBUT, there were statistically significant differences between group 1 and 2 ($p = 0.014$) but no differences were found between groups 1 and 3 ($p = 0.474$) as well as groups 2 and 3 ($p = 0.069$).

Table 24. Correlation coefficients of different levels of MG irregularity and the most relevant ocular surface parameters.

MG Irregularity						
GROUP	G1 **LOW**		G2 **MEDIUM**		G3 **HIGH**	
N	78		22		22	
	Correlation Coefficient	p	Correlation Coefficient	p	Correlation Coefficient	p
AGE	-0.087	0.447	-0.134	0.552	-0.052	0.818
SEX	-0.029	0.801	-0.014	0.949	-0.144	0.523
MG Morphological Features						
Objective MGL	-0.108	0.345	0.122	0.590	0.148	0.511
Mean Width	0.313	0.005*	0.024	0.914	0.251	0.260
Mean Length	0.134	0.243	-0.196	0.382	0.162	0.471
Number of glands	0.167	0.143	-0.230	0.303	-0.224	0.316
Quality of secretion	0.112	0.332	0.204	0.364	0.294	0.184
Ocular Surface Parameters						
OSDI	0.287	0.011*	0.154	0.494	-0.141	0.532
TFO	-0.192	0.177	-0.162	0.620	0.120	0.646
BR	-0.039	0.732	0.065	0.780	0.341	0.121
TMHk	-0.059	0.608	-0.057	0.800	0.207	0.355
NIBUT AVG	-0.207	0.069	0.114	0.613	-0.159	0.479
TBUT	-0.049	0.667	0.071	0.755	0.160	0.477
SCHIRMER	0.079	0.491	-0.003	0.990	0.003	0.990
LWE UL	-0.177	0.123	-0.237	0.287	-0.140	0.534
Corneal Staining	-0.057	0.621	0.122	0.588	-0.096	0.671

OSDI: The Ocular-surface-disease-index (score);TFO: Tear film osmolarity (mOsms/L); BR: Bulbar redness was graded automatically by K5M software; TMHk: Tear meniscus height (mm); NIKBUT-avg: average of non-invasive Keratograph tear film break-up time (seconds); LWE UL: Lid wiper epitheliopathy severity of the upper eyelid (severity score); TBUT: Tear break-up time(seconds); MG: Meibomian glands; MGL: Meibomian gland loss. (r, Spearman correlation)

***statistically significant; p- value <0.05.**

Study IV

Ocular surface temperature in DED and healthy eyes using infrared thermography

A total of 86 participants were enrolled in this study**.** Demographic data by groups are shown in **Table 25 and 26.**

Table 25. Demographic data from the control and DED group. Results expressed in mean±SD and median and IQR.

	CONTROL	DED
N	48	38
AGE (years)	39±12 40(17)	49±19 50(33)
Corporal Temperature (°C)	35.7±0.7 35.8(0.7)	35.9±0.5 35.9(0.7)
SEX (Male/Female)	14/24	25/13

DED: Dry eye disease

Table 26. Demographic data from DED group. Results expressed in mean±SD and median and IQR.

DED group		
	ADDE	**EDE**
N	16	22
AGE (years)	54±20 60 (36)	46±18 42 (29)
Corporal Temperature (°C)	36.0±0.4 35.9(0.4)	35.8±0.4 35.6(0.6)
SEX (Male/Female)	6/10	8/14

ADDE: Aqueous-deficient dry eye; **EDE:** Evaporative dry eye

No statistically significant differences were found in blink rate between healthy eyes and DED ($p = 0.1$; 11 ± 4 and 13 ± 5, respectively). Also, no statistically significant differences were found in the duration of the first and last complete IBI between healthy eyes and DED ($p = 0.1$; 4 ± 4 and 5 ± 4 seconds, respectively).

3.4.1 DED versus CONTROL

No statistically significant differences were found in MOST along 40 seconds of recording between DED and control group ($p = 0.1$; $34.74 \pm 0.78\,℃$ and $34.99 \pm 0.65\,℃$, respectively). In addition, no statistically significant differences were found in MOST at the start between DED and control group ($p = 0.1$; $34.78 \pm 0.75\,℃$ and $35.00 \pm 0.63\,℃$, respectively) as well as at the end between both groups ($p = 0.1$; $34.72 \pm 0.77\,℃$ and $34.97 \pm 0.67\,℃$, respectively). Regarding mean TER, there was no statistically significant difference between DED (49.8±39.6 x10^{-7} g/cm^2/s) and control (48.4±38.6 x10^{-7} g/cm^2/s) group ($p = 0.1$; 49.8±39.6 x10^{-7} and 48.4±38.6x10^{-7} g/cm^2/s, respectively).

Table 27 shows the comparison between DED and control group for GAP and GAP recovery in the first and the last complete IBI. There were statistically significant differences in GAP between first and the last complete IBI in the DED group ($p = 0.004$) and between DED and control group in the last complete IBI ($p = 0.004$).

Table 27. Comparison of GAP and GAP recovery between DED and control group in the first and last complete IBI. Results expressed in mean±SD and median and IQR.

		First IBI	Last complete IBI	p-value
GAP	**DED** Mean±SD Median(IQR)	0.02±0.23 0.02(0.16)	0.16±0.25 0.12(0.25)	0.004$^{\gamma}$
	CONTROL Mean±SD Median(IQR)	-0.03±0.18 -0.01(0.12)	0.01±0.19 0.02(0.20)	0.4$^{\gamma}$
	p-value **DED vs CONTROL**	0.2$^{\gamma}$	0.004$^{\sigma}$	
GAP Recovery	**DED** Mean±SD Median(IQR)	0.00±0.14 0.00(0.14)	0.02±0.27 0.01(0.17)	0.7$^{\gamma}$
	CONTROL Mean±SD Median(IQR)	0.01±0.21 -0.01(0.20)	-0.03±0.22 -0.01(0.21)	0.8$^{\gamma}$

	p-value **DED vs CONTROL**	0.8$^{\gamma}$	0.5$^{\gamma}$	

SD: Standard deviation; IQR: Interquartile range; DED: Dry eye disease. IBI: Inter- blink interval. GAP: OST difference between the end of one IBI and the beginning of the next IBI (ºC). GAP recovery: OST difference between the beginning of two consecutive IBIs in the ROI (ºC).

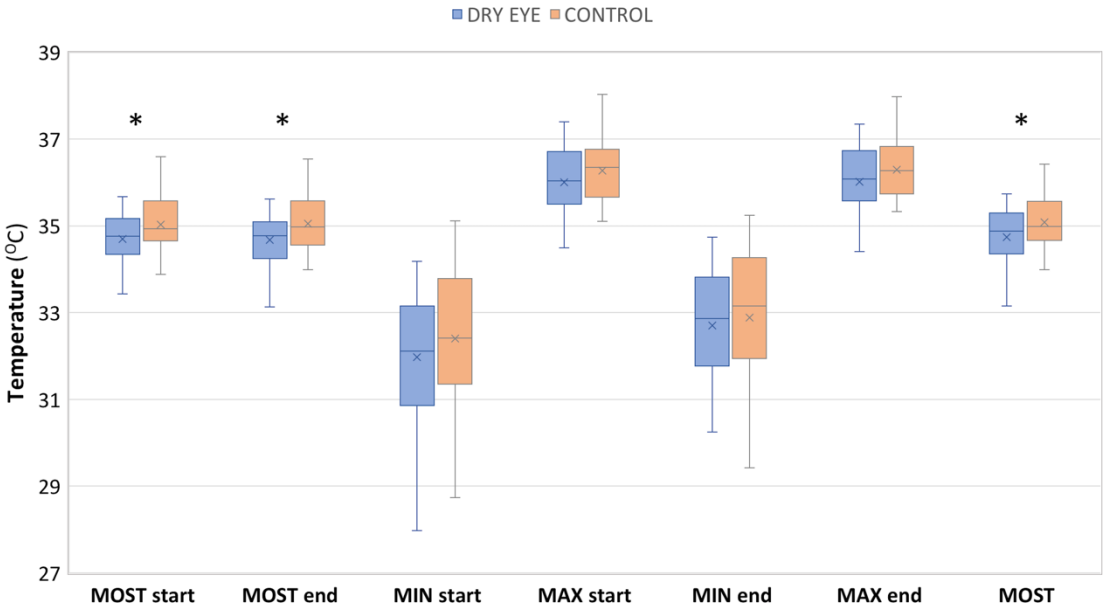
***differences statistically significant: p-value<0.05**

σ : parametric test

γ: non-parametric test

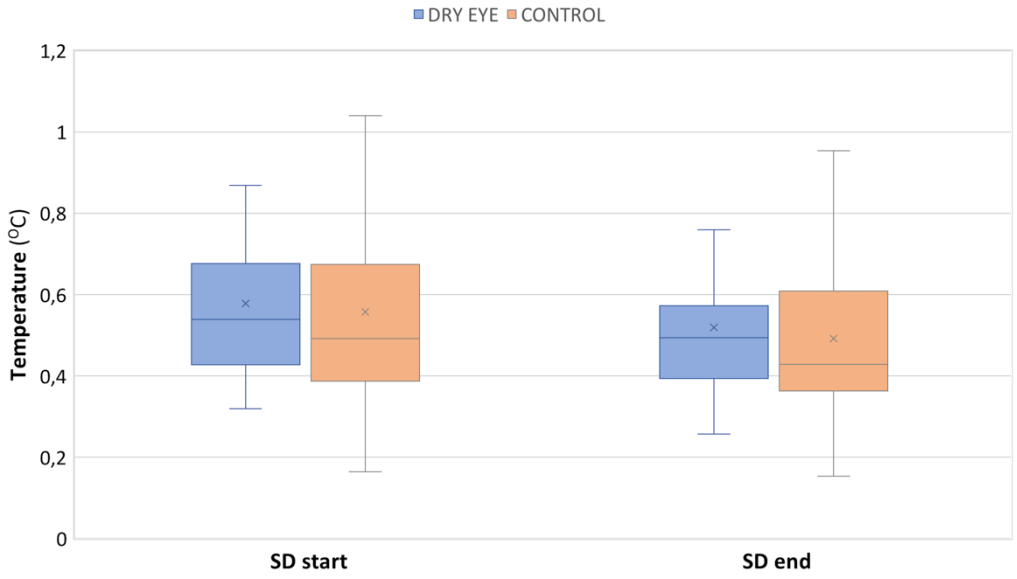
Figures 54 and 55 show the comparison between DED and control group in the first IBI. There were statistically significant differences in MOST at the start ($p = 0.03$) and at the end ($p = 0.01$) and in MOST of the IBI ($p = 0.02$) between DED and control group.

Regarding mean TER, there was no statistically significant difference between DED and control group in the first IBI ($p = 0.3$; 49.3±48.5x10^{-7} and 48.5±35.7x10^{-7} g/cm^2/s, respectively)



MOST: Mean OST of the ROI (°C). **MIN**: Minimum temperature of ROI (°C). **MAX**: Maximum temperature of ROI (°C).

Figure 54. Comparison between DED and control group in the first IBI.

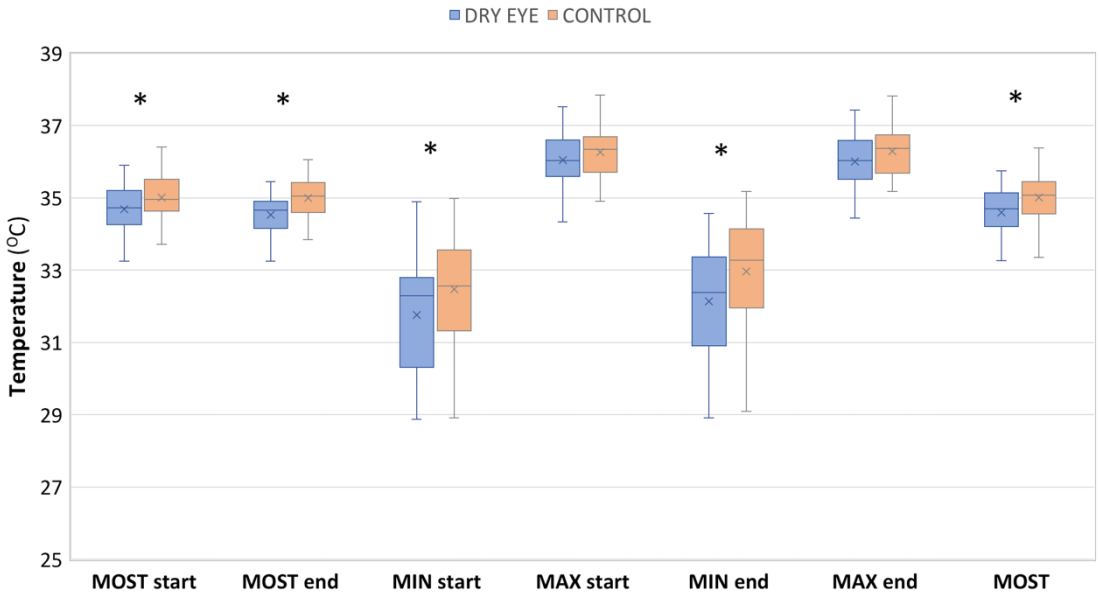


SD: Standard deviation (°C).

Figure 55. Comparison of standard deviations between DED and control in the first IBI.

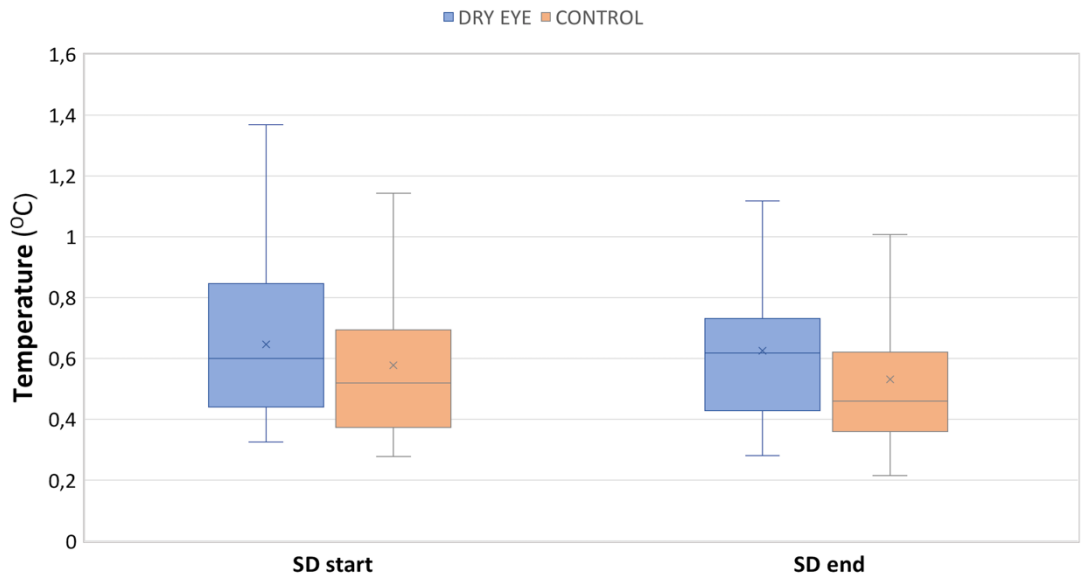
Figures 56 and 57 show the comparison of between DED and control group in the last complete IBI. There were statistically significant differences in MOST at the start ($p = 0.03$) and at the end ($p = 0.001$), MIN at the start ($p = 0.04$) and at the end ($p = 0.02$) and in MOST of the IBI ($p = 0.006$) between DED and control group.

Regarding mean TER, there was no statistically significant difference between DED and control group in the last complete IBI ($p = 0.2$; 50.0±47.7 x10^{-7} and 48.6±35.4x10^{-7} g/cm^2/s, respectively).



MOST: Mean OST of the ROI (°C). **MIN**: Minimum temperature of ROI (°C). **MAX**: Maximum temperature of ROI (°C).

Figure 56. Comparison between DED and control group in the last complete IBI.



SD: Standard deviation (°C).

Figure 57. Comparison of standard deviations between DED and control in the last complete IBI.

3.4.2 ADDE versus EDE

No statistically significant differences were found in MOST along 40 seconds of recording between ADDE and EDE group ($p = 0.1$; 34.55±0.86°C and 34.99±0.65°C, respectively). In addition, no statistically significant differences were found in MOST at the start between ADDE and EDE ($p = 0.2$; 34.62±0.84°C and 34.90±0.68°C) as well as at the end of the recording between both groups ($p = 0.1$; $34.53 \pm 0.85\,℃$ and $34.86 \pm 0.69\,℃$, respectively).

Regarding mean TER, there was no statistically significant difference between ADDE and EDE group ($p = 0.2$; 50.3± 47.5x10^{-7} and 49.6± 33.8x10^{-7} g/cm^2/s, respectively).

Table 28 shows the comparison between ADDE and EDE group for GAP and GAP recovery in the first and last complete IBI. There were statistically significant differences in GAP between first and last complete IBI in the EDE group ($p = 0.02$) and between ADDE and EDE group in the last complete IBI ($p = 0.008$).

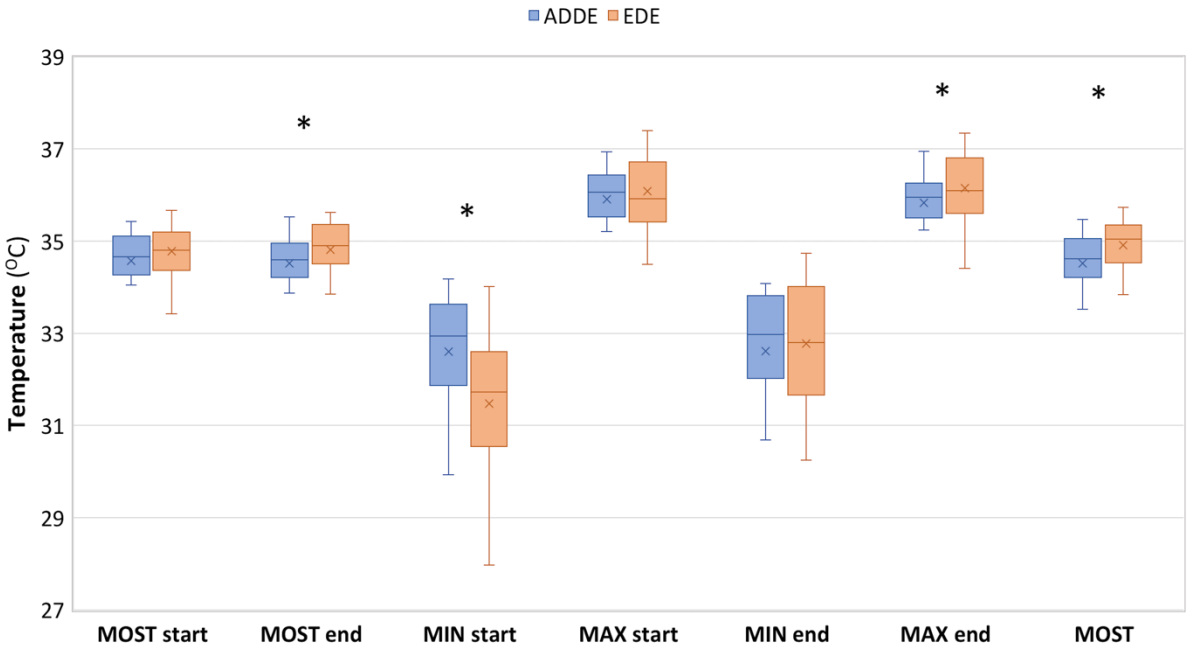
Table 28. Comparison of GAP and GAP recovery between ADDE and EDE group in the first and last complete IBI. Results expressed in mean±SD and median and IQR.

		First IBI	Last Complete IBI	p-value
GAP	**ADDE** Mean±SD Median(IQR)	0.06±0.12 0.06(0.17)	0.11±0.12 0.09(0.17)	0.1$^{\sigma}$
	EDE Mean±SD Median(IQR)	-0.02±0.28 0.02(0.16)	0.20±0.32 0.20(0.35)	0.02$^{\sigma}$
	p-value **ADDE vs EDE**	0.2$^{\gamma}$	0.008$^{\sigma}$	
GAP Recovery	**ADDE** Mean±SD Median(IQR)	-0.01±0.12 -0.01(0.10)	0.00±0.16 0.00(0.14)	0.2$^{\gamma}$
	EDE Mean±SD Median(IQR)	0.00±0.16 0.01(0.17)	0.03±0.33 0.01(0.20)	0.8$^{\gamma}$
	p-value **ADDE vs EDE**	0.8$^{\gamma}$	0.7$^{\gamma}$	

SD: Standard deviation; IQR: Interquartile range; IBI: Inter- blink interval; ADDE: Aqueous-deficient dry eye; EDE: Evaporative dry eye
GAP: OST difference between the end of one IBI and the beginning of the next IBI (°C). GAP recovery: OST difference between the beginning of two consecutive IBIs in the ROI (°C).
differences statistically significant: p-value<0.05
σ : parametric test
γ: non-parametric test

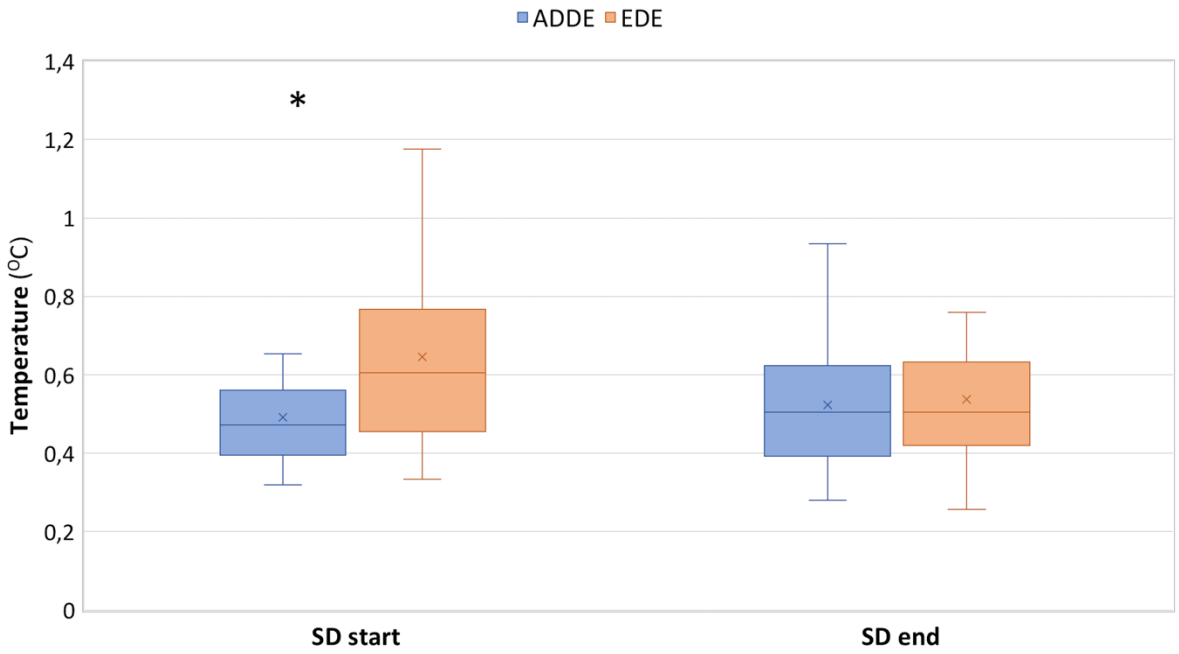
Figures 58 and 59show the comparison between ADDE and EDE group in the first IBI. There were statistically significant differences in MOST at the end ($p = 0.02$), MIN at the start ($p = 0.04$), MAX at the end ($p = 0.01$) and MOST of the IBI ($p = 0.02$) between ADDE and EDE group. In addition, a statistically significant difference was found in standard deviation at the start($p = 0.03$) but not at the end ($p = 0.5$) between both groups in the first IBI (**Figure 60**).

Regarding mean TER, there was no statistically significant difference between ADDE and EDE in the first IBI ($p = 0.5$; 49.5±63.3x10^{-7} and 49.1±34.1x10^{-7} g/cm^2/s, respectively).



MOST: Mean OST of the ROI (°C). **MIN**: Minimum temperature of ROI (°C). **MAX**: Maximum temperature of ROI (°C).

Figure 58. Comparison between ADDE and EDE group in the first IBI.

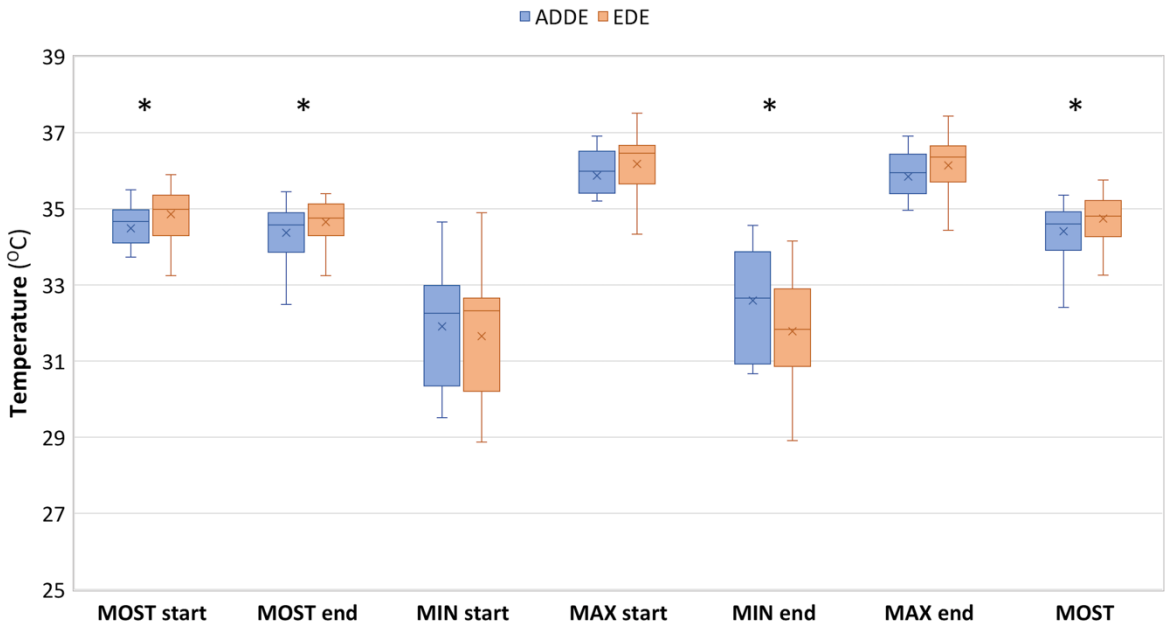


SD: Standard deviation (°C).

Figure 59. Comparison of standard deviations between ADDE and EDE in the first IBI.

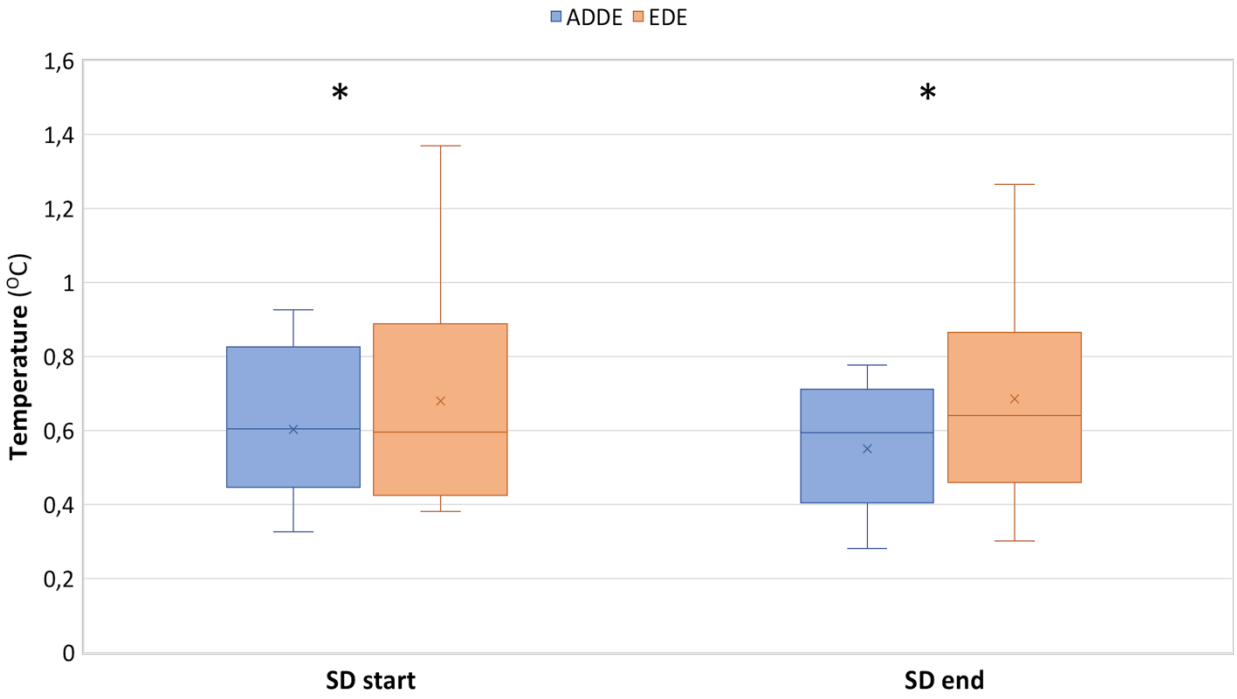
Figures 60 and 61 show the comparison between ADDE and EDE in the last complete IBI. There were statistically significant differences in MOST at the start $(p = 0.03)$ and at the end $(p = 0.003)$, MIN at the end $(p = 0.02)$ and MOST of the IBI $(p = 0.008)$ between ADDE and EDE group. In addition, statistically significant differences were found in standard deviation at the start $(p = 0.04)$ and at the end $(p = 0.03)$ between both groups in the last complete IBI (**Figure 60**).

Regarding mean TER, there was no statistically significant difference between ADDE and EDE in the last complete IBI ($p = 0.3$; 50.0±63.0x10^{-7}and 49.8±32.9x10^{-7} g/cm^2/s, respectively).



MOST: Mean OST of the ROI (°C). **MIN**: Minimum temperature of ROI (°C). **MAX**: Maximum temperature of ROI (°C).

Figure 60. Comparison between ADDE and EDE group in the last complete IBI.



SD: Standard deviation (°C).

Figure 61. Comparison of standard deviations between ADDE and EDE in the last complete IBI.

Chapter 4

Discussion and Conclusion

Study I

The effect of ageing on the ocular surface parameters

Our study findings suggest that elderly population present ocular surface changes when compared to a young population. Although the majority of the ocular surface parameters studied presented a fair correlation with age, these results give us relevant information of the ageing of the ocular surface and how it could affect the DED diagnosis. Additionally, women from this study showed more changes due to ageing than men who presented better ocular surface conditions than those of their age-matched group.

In the present research study, moderate and positive correlations (BR, LR, Corneal and conjunctival staining and TMHk, respectively) and negative correlations (TBUT and Schirmer test, respectively) were found with age. These results are in agreement with other research studies reported in the literature. Woods [283] found an increase in tear retention in patients older than 40 years that could be explained by the problems in lacrimal drainage and changes in the lid margin. Additionally, a reduction in tear secretion and TBUT have been reported in elderly patients [284,285]. Andres et al.[286] established the TBUT as predictive factor of DED problems. As well, Guillon et al.[287] found higher tear film evaporation in older patients (more in women than men) suggesting that it may be a significant contributing factor to DED in female population. Similarly, Maissa et al.[288]found that the tear film characteristics worsening with age. Another study conducted by Yeotikar et al.[196] found statistically significant associations between age and TMHk, TBUT, palpebral redness and roughness, and conjunctival staining where 185 participants (aged 25 to 66 years) were evaluated. Conversely, they found a significant negative association between TFO and age that is not in agreement with the results of our study. In addition, they did not find significant effect of age on NIBUT, tear volume (measured with phenol red) and LWE. These differences in the results might be due to the different measurements techniques, clinical devices used and the characteristics of the sample.

Likewise, MGL and MG function showed a moderate and positive correlation with age. The great amount of MGL in elderly patients and the reduction in the quality of the MG secretion are well-known and documented by several studies [178,191,289]. Moreover, an increase in lower eyelid margin thickness and in the LWE severity was observed with age. The eyelid laxity, more common in older individuals, has been reported to be associated with DED symptoms and abnormal tear parameters. It has impact on tear function that lead to a greater exposure and increased irritation [290,291], which could explain our findings regarding the increased ocular redness with age.

As highlighted by the experts of the DEWS II, there is an association between ageing and an increase in positive DED signs [22,23]. In addition, it has been reported that this increase by decade is greater with clinical DED signs compared with symptoms. Despite this, the experts suggest being cautious with the DED signs since the signs may not always be an intrinsic feature of the DED and may reflect normal ageing or other ocular surface conditions. Indeed, it is not clear how the pathological thresholds from different clinical signs could be adjusted to age [30].

Regarding subjective questionnaires, our findings showed a weak correlation (OSDI and MQ questionnaires) or no correlation (SPEED, DEQ-5 and SANDE questionnaires) with aging. Previous studies have already shown the lack of association between DED symptoms, ocular surface signs[292] and age[293]. Reduction of the tear secretion in DED patients induce inflammation and peripheral nerve damage [294]. This leads to sensitization of polymodal and mechanonociceptor nerve endings and an abnormal increase in cold thermoreceptor activity, evoking dryness sensations and pain. Prolongation of disturbances in ocular sensory pathways (molecular, structural or functional) eventually leads to dysestesias and neuropathic pain related to the eye surface[64].For example, Acosta et al.[295] conducted a study in rats and they found that the cold trigeminal neurons gradually die with aging. In the case of the human eye, a possible cause of absence or reduced dryness sensations could be explained by the aforementioned changes, justifying the lack of the association between these variables [196]. Our study findings showed that higher scores were obtained by elderly patients, but it is not consistent between questionnaires. These differences could be explained by different symptoms evaluated in each questionnaire and also by the nature of each instrument. The complexity of both central and peripheral neural mechanisms associated with ocular surface sensations and tissue homeostasis in relation to DED is still not entirely understood[64].

When the age groups were compared, statistically significant differences were found in most of the parameters assessed. These major differences were between group A (< 42 years) with B ($42 - 65$ years) and C (< 65 years), whereas the upper age groups (B and C) showed similar DED signs and symptoms. These findings highlight the differences between both age populations.

All these changes could have impact on the DED diagnosis but also in the success of several optical correction alternatives for presbyopia, such as intraocular lenses (IOLs) implantation and multifocal contact lenses (MCLs). MCLs demonstrated to be a good choice as they provide good visual quality [296–298] the desired independency from spectacles and, no less important, the aesthetic benefit (desirable mostly by women). Despite all the reported benefits, the prescription rate is still quite low. Studies as conducted by Sivardeen et al.[299] tried to determine the utility of clinical and non-clinical indicators to aid the initial selection of the optimum presbyopic CL. However, the features studied have been demonstrated to be poor indicators of the preferred MCLs type. Most of the research studies conducted about MCLs focus on visual performance and only a few of them focus on the CL interaction with the ocular surface[293]. Concerning this issue, contact lens discomfort (CLD) is one of the major issues related to CL dropout in CL wearers of all ages [300]. It is important to mention that the materials of the MCLs are the same as those that fit in young CL wearers. We believe our results about ocular surface ageing changes will provide relevant information in order to understand better the CL interaction with the eye in each population and even how these changes could impact on surgical therapies as IOLs implants or MCLs fitting. In addition, all these changes on the ocular surface would have an effect on the optical quality of the eye determined by the stability of the tear film. Consequently, it could impact on visual quality outcomes after IOL implantation or MCLs. It has also been reported that the variability in the keratometry readings is higher in patients with TFO value higher than 316 mOsm/L that could have relevant influence on the IOL power calculation [301].

Ocular surface differences between women and men were also assessed. Females had a worse ocular surface condition than men (NIKBUT-fr, NIKBUT-avg, corneal and conjunctival staining, TBUT and all questionnaires performed). A study conducted by Maissa et al.[288]found that the changes in tear film stability and lipid layer characteristics are more marked in women than men. Such a finding and the higher evaporation rate in older women aforementioned could lead to a higher corneal and conjunctival damage by environmental exposure and therefore partly explain the higher symptomatology reported by women.

In summary, this study confirms the decline in tear film with ageing and that the ocular surface of women is more affected than men. The study also demonstrated that age is a relevant factor that is needed to take into account. This is because the DED signs that are observed may not always be intrinsic features of the DED and could reflect normal ageing or another ocular surface conditions.

Study II

Relationship between MGL assessed by NIM and the ocular surface parameters and symptomatology

NIM has been proven to be a useful technique for non-invasive observation of MG morphology in order to help physicians improve DED diagnosis and treatments [169,178,302]. The aim of the study is to assess the correlation between MGL and important ocular surface parameters and it has to be determined if one or both (UL and LL) eyelid should be included for MGL assessment. This matter is quite controversial since different studies with different aims have considered both or only one eyelid. Routinely, LL is the most commonly assessed due to its accessibility which provokes less discomfort to the patient. It is believed that comparable outcome can be expected for MGL assessment choosing one or two eyelids, as UL and LL MGL seem to be significantly correlated [190]. Dogan et al.[303] assessed meibography images of 30 patients and proposed to evaluate only the UL for MGL assessment because of its correlation with TBUT and better inter-examiner agreement on MGL. On the other hand, Finis et al.[226] performed a retrospective analysis of 128 patients and found a strong correlation between meiboscores of the UL and the LL as well as with the total meiboscore. This suggests that MGL assessment based on the evaluation of the LL might be enough for the clinical routine. In the present study a positive and fair statistically significant correlation between MGL of UL and LL was found. However, there was no agreement as revealed by the Kappa statistic, suggesting that, despite there exist a relationship between MGL of both eyelids there is a bias in their meiboscore.

Our results are in accordance with the study done by Pult et al.[190], who suggested the MGL assessment of both eyelids. Therefore, in our study both eyelids were taken into account in order to assess MGL and participants were classified into five groups according to the *total meiboscore* [178].

DED symptomatology was evaluated using OSDI and SPEED questionnaires in the current study. No statistically significant differences were found in DED questionnaires among different MGL groups. However, as Table 4 shows, the symptomatology score for group 5, in both questionnaires, is clinically higher than for the other groups. It has been reported that an MGL of >32% is likely to be accompanied with associated detectable clinical symptoms [179]. Similarly,

several studies have found correlation between MGL and OSDI scores [196,304]. However, other studies have not found any correlation between MGL and OSDI scores [303,305] or SPEED questionnaire [306].

Regarding MGL and its correlation with other clinical parameters, statistically significant differences were found in TFO between group 5 and groups 1,2 and 3. Besides, statistically significant differences were found in corneal staining as well as conjunctival staining between group 5 and groups 1 and 2. These results are in agreement with those obtained by Feng et al.[305] who found a positive correlation between corneal staining and MGL in DED patients. Previous studies have suggested that when the amount of MG reduced, the secretion of MG decreases. This might induce tear film homeostasis loss and greater tear film evaporation which could lead to surface epithelial damage, and disturbance of the glycocalyx and goblet cell mucins [307]. In the present study no differences were found in TBUT and Schirmer test among different MGL groups. These findings are in accordance with other studies that did not find any correlation between MGL and the tear film parameters [308] or a low correlation was found [305]. Nevertheless, Arita et al. [309] studied a population with MGD and found that Schirmer test was positively correlated with the *meiboscore.* Thus, patients with less amount or damaged MG would have an increase of fluid that may compensate the decreased function of the lipid layer. These findings suggest that these ocular surface parameters could be affected by MGL in patients who suffer from MGD. The glandular loss may exacerbate the signs of MGD in comparison with those who only present MGL without other ocular condition. No statistically significant differences were found in any of the 5KM parameters among MGL groups except for BR. Recently, Ji et al.[310] found a correlation between MGL grade and NIKBUT-avg, NIKBUT and LLT in patients with DED and MGD. This discrepancy between studies could be due to the study population including only subjects with DED and MGD.

Our findings suggest that a MGL higher than 50% is accompanied by signs of increased osmolarity, redness and staining of the ocular surface. However, it is important to highlight that in our study the mean age of the participants was higher in the groups with higher MGL. Therefore, it is not possible to ascertain if these signs in ocular surface parameters are due to MGL or to age. The great influence of aging on MG morphology and function is well-known and documented in the literature [178,190,308,311]. In order to address this point, the relationship between MGL and the ocular surface parameters was assessed considering age as a covariant. When it was performed, only the corneal staining was correlated with MGL. These results emphasize the

influence of ageing in the MG morphology but also in several ocular surface parameters such as corneal staining, LLT or tear volume among others [288,311]. The importance of participants age when performing research studies focused on the ocular surface has been previously reported [311] and pointed out. Thus, in order to assess the real impact or influence of the MGL on the ocular surface parameters (which are influenced by age), it would be necessary to compare matched age groups. For example, MGL has been found to be positively correlated with meibum quality suggesting an impaired MG function when MGL increase [141,310,312]. The present study has shown that when age is covariant, the relationship between MGL and meibum quality is absent, indicating that meibum quality could be decreased either because of a higher amount of MGL or because it is naturally decreased with aging. These findings suggest that different thresholds for defining abnormal ocular and tear film surface parameters should be considered according with the age. Next steps should be focused on determining the normal values of different parameters for each age range.

In summary, this study suggests that a MGL higher than 50% is accompanied by signs in the ocular surface. In the light of the findings, future studies must consider age due to its great influence on the MG morphology. Also, to compare age-matched groups in order to know the contribution of the MGL on the ocular surface as well as establishing valid cut-off values for DED diagnosis.

Study III

Relationship between new objective MG morphological parameters and the ocular surface parameters

The assessment of the MG morphology is currently receiving much attention due to the impact of the DED and, particularly of the MGD worldwide. Since the introduction of the NIM [178] in the clinical setting, both researchers and eye care practitioners have been able to obtain useful information of the MG morphology in a fast and patient-friendly way in order to perform a better DED diagnosis. As previously explained, MGL has been the most reported MG feature observed through NIM [166] which has served as indicator of severity of the condition. Typically, it has been graded subjectively by researchers and eye care practitioners but, in the last few years more objective methods have been suggested. Nowadays, the MG morphology assessment is advancing towards to an automatic approach due to its numerous benefits. One of these advantages is obtain more reliable information because the measurement is not dependent of the user and, on the other hand, it gives the possibility to assess other morphological features apart from the MGL (such as the length, width, shape, contour and tortuosity) [169,179,313]. Despite this, the automatic analysis of the meibography images based on imaging processing appear to be challenging. Many factors can influence this analysis such as specular reflections, low contrast, artefacts, defocused areas and as well, non-uniform illumination[314]. To date, several automated algorithms based on image processing have been proposed to assess MG morphology. In 2012, Koh et al.[192] introduced a computational method to detect the length and width of the MG without requiring any input from the user. They classified a total of 55 meibography images as *healthy* and *non-healthy*, obtaining a specificity of 96% and a sensitivity of 98%. Despite this, the algorithm failed to classify the images, which considered as "intermediate". According to the authors, it is necessary to evaluate additional morphological features such as MG distortion. Later on, in 2013, Celik et al. [314]developed an automated method that allowed the identification of gland and inter-gland regions (two-class segmentation) of 131 meibography images. As well, it allowed the classification of the meibography images into *healthy*, *intermediate* and *unhealth* with an accuracy of 88%. However, this was designed to analyse only the UL. More recently, in 2016, Koprowski et al.[194,195] proposed a full automated algorithm tested with a total of 172 meibography images (from the UL and LL) of 55 DED and 31 healthy subjects which were

compared with the results of an expert clinician. The subjects of this work were classified into three groups: healthy, at-risk and affected. The study showed reproducible results obtaining a sensitivity of 99.3% and specificity of 97.5% in the MG diagnosis.

The automated algorithm used in the present study provides a three-class classification and it is able to differentiate between gland, inter-gland and areas of MGL. Indeed, it provides an objective estimation of the MGL area and as well, other morphological parameters of MG such as mean length, mean width, number of glands and especially, the irregularity. To the extent of our knowledge, this is the first attempt to quantify the irregularity of the MG using an automated method. The assessment of this morphological parameter in particular could provide valuable information about the progression of the MGD [141,180].

Currently, limited information is available regarding the relationship between objective MG morphological parameters and the ocular surface. For example, Ban et al.[191] conducted a morphometric assessment using NIM and studied the relationship between the MG morphology and the ocular surface. In order to accomplish this, they evaluated the mean length of the MG ducts (five central MG ducts), percentage area of MG acini and number of glands dropouts of 37 healthy subjects (both eyelids) analysed semi-automatically with ImageJ software. Both mean MG duct length and percent area of MG acini were negatively correlated with age ($r = -0.485$ and $r = -0592$, respectively) while the number of gland dropouts showed positive correlation with age ($r = 0.518$). As well as previous studies, the age is a relevant factor regarding the MG morphology, having an impact also in the length and the percentage of MG acini in this study [178,304]. In addition, they found that the mean length of the MG ducts in the UL and LL showed negative correlations with the meibum secretion and the corneal staining score. As well, the percent area of MG acini in the UL showed a positive correlation with TBUT and negative correlations with the tear film lipid layer interferometry and meibum secretion. Instead, the present study found significant positive correlations between THMk and BR with the objective MGL and negative correlations with the objective mean length of the MG. This finding makes sense as the objective MGL and length are related (reduced MG length may indicate that part of the MG disappears). Arita et al.[309] previously reported that an increased tear fluid could compensate the decreased function of the lipid layer. Additionally, this change in the tear film could lead to an inflammation of the ocular surface, represented by the ocular redness.

Despite the previous results, it is important to note that the majority of the objective MG morphology parameters (objective MGL, mean width and mean length) showed a significant

correlation with age and no correlation with sex. Indeed, when age included as a covariant, it is observed that there is no relationship between these three objective MG morphology parameters and the most relevant ocular surface parameters. In other words, the influence of these MG morphological parameters on the ocular surface parameters could be smaller in comparison to the influence provided by age. Therefore, these findings highlight the relevance of the age as a factor to study the influence of MG morphology on the ocular surface integrity.

Beyond the MG morphological parameters aforementioned, the other morphological parameter to analyse in this study was the irregularity of the MG (most commonly named as *tortuosity* or *distortion*). According to Mathers et al.[157], MG distortion appears in the first stage of morphological changes of the MG. In fact, it has been observed in patients with perennial AC [212] as well as in patients with CL-related allergic conjunctivitis (CLAC) [315]. In the first case, patients with perennial AC who presented MG distortion showed higher meibum score in comparison to those with perennial AC that no showed MG distortion. Similarly, in the second case, CL wearers with CLAC showed impaired MG function in comparison to CL wearers without CLAC. These findings suggest the influence of the MG distortion upon seeing the MG function diminished. According to the authors, it is suggested that the AC, not the CL wear, is associated with MG distortion. Indeed, it is believed that the MG distortion could be due to inflammatory changes in the conjunctival tissue that might induce pressure on the MG in the tarsus [316,317]. On the other hand, MG distortion has been observed in CL wearers (both soft and rigid CL) [206] and the reason behind it is thought to be the chronic friction provoked by the CL. Due to the lack of information about the MG distortion, more investigations are needed about its origin. It is relevant to mention that both MGL and MG distortion make reference to different aspects of the MG morphology, so it is expected not to find a relationship between them (the first one reflects the loss of area and the second the changes in shape) [178]. Indeed, in the present study, these two MG morphology features are not well related between irregularity, number of glands and quality secretion. On the other hand, the irregularity showed a positive correlation with the length and the width of the MG. This indicates that when the irregularity increase, the width and the length increase too. This finding is interesting because it is expected or believed that when it is observed an increase of irregularity is led to an increase in the width and a decrease in the length of the MG since MG tends to shrink itself. Nevertheless, this was not observed in our study. The reason that these results are unknown could be related to the automatic calculation and mathematics principles used in the algorithm. Further studies are needed to clarify this matter.

As well as the MGL, the assessment of the MG distortion is performed subjectively, usually as “present” or “absent”[313] or even, for example, grading it from 0 to 2 (grade 0: no distortion of the MG; grade 1: 1–4 MG with distortion $> 45^{\circ}$; grade 2: more than five MG with distortion $> 45^{\circ}$).[315].

To the best of our knowledge, this is the first study that shows an objective attempt to report the irregularity of the MG. In addition, it was studied its relationship with some of the most relevant ocular surface parameters. As observed, it had no correlation with any ocular surface parameter, even with DED symptomatology. For this reason, we are led to ask ourselves if different levels of irregularity (participants were classified into *low, medium* or *high* irregularity) could be related to these ocular surface parameters. As well, no correlations were found except for group 1 (*low*) who exhibited a fair and positive correlation with OSDI and also with the width of the MG. Notwithstanding, the results need to be carefully interpreted since the groups of irregularity are not homogeneous and there is a big difference between group 1 and the other two groups.

In summary, as found in the previous chapter, the majority of the objective MG morphology parameters obtained with the automated algorithm are highly influenced by age. The influence provided by those MG morphology parameters on the ocular surface could be smaller in comparison to the influence provided by age. Regarding MG irregularity, it may represent the prodromal stage before losing the MG and as long as the gland is not atrophied, it will be functioning, and the ocular surface will not be affected. However, this hypothesis needs to be confirmed with a prospective study to assess the MG irregularity changes in time and its natural course as well as to clarify the role of this morphometric feature on the DED context.

Study IV

Ocular surface temperature in DED and healthy eyes using IR thermography

The IR thermography has been widely used during the last decades to assess non-invasively the tear film, both static and dynamically. First, Morgan et al.[264] emphasized the potential of this technology for tear film diagnosis and later on, Purslow et al.[262] demonstrated the existing connection between the tear film and OST. The current study provides more information about the OST changes (both DED and healthy eyes) and the effect of blinking by assessing several OST metrics and specifically the IBI. Several research studies have demonstrated that DED subjects present cooler OST and faster cooling rates compared to healthy subjects [257,318]. For example, Craig et al.[319] found that DED patients have a significantly lower temperature GCC than controls. Tan et al.[258] found lower temperatures in DED subjects after 5 and 10 seconds of eye-opening (GCC, MOST, minimum and maximum temperature and different areas of the eye). Kamao et al.[259] also observed greater decrease in OST in DED eyes at 10 seconds after eye-opening and, in addition, they found that eyes with a shorter TBUT are more likely to have a decrease in the OST. Using a remote sensor thermometry, Singh and Bhinder[320] also found lower mean OST value in DED eyes compared to that found in normal subjects. On the other hand, a study conducted by Morgan et al[264] found an increased MOST in DED patients ($32.38 \pm 0.69\,℃$) in comparison to controls ($31.94 \pm 0.54\,℃$) that could be attributed to inflammation or increased hyperemia within the DED group participated in their study [263,321]. Nevertheless, other research studies reported no significant difference in MOST immediately after eye open between DED and normal subjects [322,323]. In the present study, DED showed a slightly cooler OST in comparison to the control group in MOST along 40 seconds of recording and as well as in MOST at the start and at the end between both groups. However, the results were not statistically significant. Despite this, when the IBI of both groups were assessed and compared, statistically significant differences were found. In the first IBI was found statistically significant differences in MOST at the start and at the end and MOST of the IBI. On the other hand, in the last complete IBI was found, apart from the aforementioned OST metrics, in the MIN at the start and also at the end. These findings suggest that after a period of natural blinking, the minimums temperatures are affected whereas the maximums temperatures remain almost stable in both

groups. However, these changes in the minimums temperatures between IBIs could be attributable to the different conditions of the measurement. The first IBI was measured just after the blink and after the participant remained with the eyes closed for 3-4 seconds. This measurement is not highly influenced by the exposure of the ocular surface and could maintain a warming effect of the eyelid in the last blink before opening the eye. On the other hand, the last complete blink could be more influenced by the ambient surrounding or by partial blinks. In fact, partial blink is known that affect the stability of the tear film and its distribution in the inter-blink period [324]. Hence, it is possible that first IBI is not a representative IBI and may provide a false-positive. Other reason could be due to the tear film dynamic since it is known that tear film has a complete cycle with four different parts [325]. Indeed, the tear film is constantly changing by undergoing a formation (build-up) phase directly after a blink, a fairly stable inter-blink phase and ultimately tear film destabilisation that can lead to tear film break-up in subjects with DED or when the eye is left open for a long period of time. In addition, it has been reported that tear film lipids tend to be relatively stable from blink to blink [326], therefore, maybe we are comparing different parts of the blinking cycle lead to these differences. Further studies using natural blinking are needed in order to elucidate this point and obtain more information about the natural dynamic of the blink.

Furthermore, a significant difference was found in GAP between groups in the last complete IBI, being higher in the DED group. It demonstrates that DED eyes cooling quicker than healthy eyes and thanks to the blink action the OST of the DED eyes can be restored (GAP recovery remains stable). Zhang et al.[327] reported that eyelids affect the OST since the eyelid provides thermal pulses to the ocular surface. In addition, Deng et al.[328] using theoretical models of thermal dynamics explored the temperature profiles during the partial blink for the tear film and the simulation revealed that lid motion had a significant effect on corneal temperature during the blinking and for a short period after the blink. In their study, the temperature GCC for a full blink remains periodic over multiple blink cycles. In the case of partial blinks, there is a noticeable reduction of the peak temperature for each in comparison to the full blinks. This finding suggests that the partial blink may affect the thermal stability. As previously reported by Shen[329], incomplete blinking is frequently found in patients with DED, therefore the blink could be more determinant in DED eyes.

The TER is a relevant parameter in the study of the tear dynamics[266]. Previously, NIBUT has been negatively correlated with evaporation rates[144], indicating that an increased evaporation causes more rapid thinning of the tear film between blinks leading to faster break-up. Indeed, a recent study found evidence that ocular surface cooling and tear film break-up are associated and suggest that a common physical force such as tear film evaporation is acting in both processes[330]. Several studies have reported an increased TER in subjects with DED [164,319,331,332] (ADDE, EDE and mixed) while others did not found the same results [270]. In this study, no significant differences were found in TER between DED and control groups nor between ADDE and EDE groups. In the literature is possible to find a wide range of TER values (between 0.02 to 29 x 10^{-7} g/cm^2/s in human eyes) for healthy and DED eyes that depends on the ambient temperature, relative humidity and also the technology used in each study [270]. TER values obtained in the current study (up to 40 x 10^{-7} g/cm^2/s) lie out of the range reported in previous studies but it is well-known that this parameter is highly influenced by external factors as ambient temperature and humidity [333,334].For example, Petznick et al.[335] measured TER values in healthy subjects using an infrared thermal camera at $30\,℃$ under two different percentages of relative humidity (45 and 65) and found values around 13.5 and 11.8 x 10^{-7} g/cm^2/s, respectively. In addition, Tan et al.[266]found a TER of 25.38 ± 5.63 x 10^{-7} g/cm^2/s at 70% of relative humidity in healthy subjects. As observed, there is a great discrepancy among studies and the reason is unclear. However, it could be related to the environmental conditions during the measurements or due to small changes in the focus of the image that could introduce an error in the measurements since the thermography is very sensitive to the focusing. Future studies are needed to corroborate these findings.

While OST differences between DED and normal subjects is well-documented, only a few studies have evaluated the thermal differences between different DED types[336,337]. Abreu et al.[336] found lower mean initial OST in ADDE patients (GCC, nasal and temporal conjunctiva) in comparison to EDE, SS and control. Besides, ADDE patients showed the most substantial heat loss over the 5 seconds of IBI ($-0.97\,℃$). On the other hand, more recently, Matteoli et al.[337] found that EDE patients have the maximum temperature variation ($-0.75\,℃$) and the highest average cooling rate. In this study, no statistically significant differences were found in MOST along 40 seconds and MOST at the start and at the end of the recording between ADDE and EDE. Despite this, when the first and the last complete IBI between both groups were assessed, statistically significant differences were found. Indeed, in the first IBI was observed in MOST at the end, MIN

at the start, MAX at the end and MOST of the IBI whereas in the last complete IBI was found in the MOST at the start and at the end, MIN at the end and MOST in the IBI. Our findings suggest that the ADDE group showed cooler OST but the OST variability was greater in the EDE group. In addition, EDE showed higher GAP which lead to quicker cooling in comparison to ADDE. On the other hand, both groups showed no significant changes in GAP recovery which could mean that the eyelid provides a warming effect of the ocular surface in each blink. As well as the comparison between DED and control, the assessment of the IBI has provide more information about the OST changes between groups. Apart from the methodology used to assess the OST, the inconsistences in the results among different studies could be also due to the different DED diagnosis criteria established or even to the different degree of DED severity.

In summary, the evaluation of the thermal behaviour during the IBI could provide useful information about the thermal changes of the ocular surface in DED and healthy eyes.

Chapter 5

Summary

The following is a summary of the conclusions taken from the four performed studies:

1) *Age is a relevant factor that is necessary to take into account when the ocular surface is assessed since the DED signs that are observed may not always be intrinsic features of the DED and could reflect normal ageing or another ocular surface conditions.*

2) *The assessment of the MGL subjectively using the meiboscore showed that a MGL higher than 50% is accompanied by signs of increased osmolarity, redness and staining of the ocular surface. Despite this, when age was included as a covariant only the corneal staining was correlated with MGL, emphasizing the influence of ageing in the MG morphology but also in several ocular surface parameters. Overall, these findings suggest that age-matched groups should be compared in order to know the contribution of the MGL on the ocular surface as well as establish valid cut-off values for DED diagnosis.*

3) *It is possible to obtain further information regarding the MG morphology such as the area of loss, length, width and irregularity from a 2-D meibography image using an automated algorithm based on image processing.*

4) *The majority of the objective MG morphology parameters obtained using an automated algorithm were also influenced by age, thus age-matched groups should be compared in order to know their contribution on the ocular surface integrity.*

5) *To the best of our knowledge, it is the first study that shows an objective attempt to report the irregularity of the MG as well as its relationship with relevant ocular surface*

parameters. According to the findings, the role of the MG irregularity as a clinical parameter is not clear, it may represent the prodromal stage before losing the MG.

6) *The NIM is a useful tool for the assessment of the morphology of the MG. However, in order to study the effect of changes in the gland morphology on the ocular surface, it is essential to consider age.*
7) *The evaluation of the IBI provided further information about the thermal changes on the ocular surface of healthy and DED subjects.*

5.1 FUTURE STUDIES

- The MG structure was assessed only at one point in time in the present study. Long-term prospective serial imaging of the MG would allow further understanding of the natural history of MG atrophy as well as other MG morphology features such as MG irregularity and its time course in our natural life cycles.
- The influence of age on the MG morphology and the ocular surface has been highlighted in this work. Future studies must consider age due to its great influence on the MG morphology. Also, to take into account on comparing matched-age groups in order to know the contribution of the MGL on the ocular surface as well as establishing valid cut-off values for DED diagnosis. This could help clinicians in order to carry out a better DED diagnosis.
- Currently, the study of the MG morphology involves an automatic approach in order to obtain more reliable information and reduce the time of the clinical evaluation. Future studies should incorporate an automatic method to assess the MG morphology. In addition, to study more deeply on the information that this approach could give the clinicians in order to help on the early prevention of ocular surface diseases or conditions such as MGD or DED.
- The IR thermography has shown to be a promising technology to assess the tear film dynamics. Future studies need to incorporate this technology in order to assess the tear film with other ocular surface conditions. The MGD field could be interesting to in order to explore the temperature differences between patients who show a low, medium and high amount of MGL or even between different levels of irregularity and to study its influence on the ocular surface temperature.

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analysis in infrared images of meibography. J Optom 2013;6:194–204.

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EPILOGUE

1. SCIENTIFIC DISSEMINATION

The proposed dissertation is a far-reaching work focused on the use of IR technology for MG structure assessment and thermal stability of the tear film which resulted in the following publications carried out for this doctoral thesis:

Scientific articles

- **The effect of ageing on the ocular surface parameters.**
  Authors: **Laura Rico-del-Viejo,** Amalia Lorente-Velázquez, José Luis Hernández-Verdejo, Ricardo García-Mata, José Manuel Benítez-del-Castillo, David Madrid-Costa. Cont Lens Anterior Eye. 2018 Feb; 41:5-12. doi: 10.1016/j.clae.2017.09.015.
- **The influence of Meibomian gland loss on ocular surface clinical parameters.**
  Authors: **Laura Rico-del-Viejo,** José Manuel Benítez-del-Castillo, Fernando Javier Gómez-Sanz, María García-Montero, Clara Llorens-Quintana, David Madrid-Costa. Apr 10. pii: S1367-0484(18)31061-0. doi: 10.1016/j.clae.2019.04.004
- **Interaction between presbyopia correction and dry eye disease: A review.**
  Authors: **Laura Rico-Del-Viejo,** Irene Martínez-Alberquilla, Pablo de Gracia, Javier Ruiz- Alcocer, James Wolffsohn, David Madrid-Costa. J Ophthalmol. 2019 **(under review in Journal of Ophthalmology).**
- **Drop Out the Area: on the importance of additional parameters when assessing meibomian glands condition.**
  Clara Llorens-Quintana, **Laura Rico-del-Viejo**, Piotr Syga, David Madrid-Costa, Robert Iskander. **(under review in Cornea Journal).**
- **A novel automated approach for infrared-based assessment of Meibomian gland morphology.**
  Authors: Clara Llorens-Quintana, **Laura Rico-del-Viejo**, Piotr Syga, David Madrid-Costa, and D. Robert Iskander. **(under review in TVST).**
- **Effect of blinking rate on tear film optical quality with different contact lenses.**
  Authors: María García- Montero, **Laura Rico-del-Viejo**, Irene Martínez-Alberquilla, José Luis Hernández-Verdejo, Amalia Lorente-Velázquez, David Madrid-Costa**. (under review in Journal of Ophthalmology).**

- **Repeatability of non-invasive Keratograph 5M measurements associated with contact lens wear.**

  Authors: María García- Montero, **Laura Rico-del-Viejo**, Amalia Lorente-Velázquez, Irene Martínez-Alberquilla, José Luis Hernández-Verdejo, David Madrid-Costa**.** Eye Contact Lens. 2019 Mar 29. doi: 10.1097/ICL.0000000000000596**.**

- **Randomized crossover trial of silicone hydrogel contact lenses.**

  Authors: María García-Montero, **Laura Rico-del-Viejo**, Clara Llorens-Quintana, Amalia Lorente-Velázquez, José Luis Hernández-Verdejo, David Madrid-Costa**.** Cont Lens Anterior Eye. 2018 Dec 23. pii: S1367-0484(18)30943-3. doi: 10.1016/j.clae.2018.12.006.

- **Comparison of the impact of Nesofilcon A hidrogel contact lens on the ocular surface and comfort of presbyopes and non presbyopes wearers.**

  Authors: Amalia Lorente-Velázquez, Maria García-Montero, Fernando Javier Gómez Sanz, **Laura Rico-Del-Viejo**, Jose Luis Hernández-Verdejo, David Madrid-Costa. Int J Ophthalmol. 2019 Apr 18;12(4):640-646. doi: 10.18240/ijo.2019.04.19

- **Nonsurgical Procedures for Keratoconus Management – Review.**

  Authors: **Laura Rico-Del-Viejo,** Maria García-Montero, Jose Luis Hernández-Verdejo, Santiago García-Lázaro, Fernando Javier Gómez Sanz, Amalia Lorente-Velázquez. J Ophthalmol. 2017; 2017:9707650. doi: 10.1155/2017/9707650

Oral Communications

- **The role of Meibomian gland atrophy on contact lens discomfort.**

  Authors: **Laura Rico-del-Viejo,** Nina Tavberidze, María García-Montero, Amalia Lorente-Velázquez, José Luis Hernández- Verdejo, David Madrid-Costa

  14° International Conference of Optometry and Vision Science (CIOCV'2017). University of Minho, Braga, Portugal. April 22-23th, 2017

- **Meibomian gland dropout as a predictor of the success of the multifocal contact lens fitting: A pilot study.**

  Authors: **Laura Rico-del-Viejo,** Nina Tavberidze, Amalia Lorente-Velázquez, José Luis Hernández-Verdejo, David Madrid-Costa

  40th BCLA Clinical Conference & Exhibition. ACC Liverpool, Liverpool, UK. June 9 – 11thJune 2017

- **Impact of a daily hydrogel contact lens with higher water content on the ocular surface of non-presbyopic and presbyopic population.**

  Authors: **Laura Rico-del-Viejo,** Nina Tavberidze, Amalia Lorente-Velázquez, José Luis Hernández-Verdejo, David Madrid-Costa

  40th BCLA Clinical Conference & Exhibition. ACC Liverpool, Liverpool, UK. June 9 – 11thJune 2017

- **Impacto de la pérdida de glándulas de meibomio (drop-out) en la película lagrimal y la superficie ocular.**

  Authors: **Laura Rico-del-Viejo**, Nina Tavberidze, María García-Montero, Fernando Gómez- Sanz, Amalia Lorente-Velázquez, José Luis Hernández-Verdejo, David Madrid-Costa.

  25° Congreso Internacional de Optometría, Contactología y Óptica Oftálmica (OPTOM) Madrid, Spain. April 13-15th, 2018

- **Envejecimiento y género, ¿Cómo influyen en la superficie ocular?**

  Authors: **Laura Rico-del-Viejo**, Nina Tavberidze, María García-Montero, Fernando Gómez- Sanz, Amalia Lorente-Velázquez, José Luis Hernández-Verdejo, David Madrid-Costa.

  25° Congreso Internacional de Optometría, Contactología y Óptica Oftálmica (OPTOM) Madrid, Spain. April 13-15th, 2018

- **Meibomian glands integrity and its relationship with the ocular surface.**

  Authors: **Laura Rico-del-Viejo**, Irene Martínez-Alberquilla, María García-Montero, Fernando Gómez- Sanz, Amalia Lorente-Velázquez, David Madrid-Costa.

  VI International Congress of Research in Retina and Vision. Inst. Investigaciones Oftalmológicas Ramón Castro Viejo, Madrid, Spain. June 29-30th, 2018

- **Addition and pupil size effect on the visual performance of a novel extended-depth-of-focus contact lens and a center-near design**

  Authors: Javier Ruiz-Alcocer**,** Irene Martínez- Alberquilla, **Laura Rico-del-Viejo,** David Madrid-Costa.

  16° International Conference of Optometry and Vision Science CIOCV'2018. Altice Forum Braga, Braga, Portugal. May 4-5th

- **Ocular surface temperature in dry eye and healthy eyes using non-contact infrared thermography**

  Authors: Javier Ruiz-Alcocer**,** Irene Martínez- Alberquilla, **Laura Rico-del-Viejo,** David Madrid-Costa.

  16° International Conference of Optometry and Vision Science CIOCV'2018. Altice Forum Braga, Braga, Portugal. May 4-5th

Poster Presentations

- **Non-contact meibography: The relevance of the assessment of the meibomian glands structure in the clinical setting.**

  Authors: **Laura Rico-del-Viejo,** Nina Tavberidze, José Manuel Benítez-del- Castillo, Amalia Lorente-Velázquez, Asunción Peral, David Madrid-Costa.

  13° International Conference of Optometry and Vision Science (CIOCV'2016). University of Minho, Portugal. April 23th-24th, 2016

- **Impact on the ocular surface of a new daily hidrogel contact lens with high water content.**

  Authors: **Laura Rico-del-Viejo,** Nina Tavberidze, Amalia Lorente-Velázquez, José Luis Hernández-Verdejo, David Madrid-Costa.

  8th Tear Film & Ocular Surface Conferences (TFOS). Le Corum, Montpellier, France. September 7th-10th, 2016

- **Effects of contact lens wearing on tear film and ocular surface of presbyopes population.**

  Authors: **Laura Rico-del-Viejo,** Javier Ruiz-Alcocer, Nina Tavberidze, Amalia Lorente-Velázquez, José Luis Hernández-Verdejo, David Madrid-Costa.

  8th Tear Film & Ocular Surface Conferences (TFOS). Le Corum, Montpellier, France. September 7th-10th, 2016

- **Infrared Meibography as a tool for Meibomian gland Dysfunction classification.**

  Authors: **Laura Rico-del-Viejo,** Nina Tavberidze, María García-Montero, Amalia Lorente-Velázquez, José Luis Hernández- Verdejo, David Madrid-Costa.

  14° International Conference of Optometry and Vision Science (CIOCV'2017). University of Minho, Braga, Portugal. April 22-23th, 2017

- **Comparison of temporal ocular surface temperature changes induced by different contact lens materials.**

  Authors: José Luis Hernández- Verdejo**, Laura Rico-del-Viejo,** María García-Montero , Nina Tavberidze, María García-Montero, Amalia Lorente-Velázquez, José Luis Hernández- Verdejo, David Madrid-Costa.

  14° International Conference of Optometry and Vision Science (CIOCV'2017). University of Minho, Braga, Portugal. April 22-23th, 2017

- **Comparison of tear film optical quality dynamics in contact lens wearers.**

  Authors: María García-Montero, **Laura Rico-del-Viejo,** Nina Tavberidze, Amalia Lorente-Velázquez, José Luis Hernández- Verdejo**,** David Madrid-Costa.

  14° International Conference of Optometry and Vision Science (CIOCV'2017). University of Minho, Braga, Portugal. April 22-23th, 2017

- **Impact of blinking frequency on tear film optical quality dynamic in contact lens wearers.**

  Authors: María García-Montero, **Laura Rico-del-Viejo,** Nina Tavberidze, Amalia Lorente-Velázquez, José Luis Hernández- Verdejo**,** David Madrid-Costa.

  14° International Conference of Optometry and Vision Science (CIOCV'2017). University of Minho, Braga, Portugal. April 22-23th, 2017

- **Impact of blinking frequency on the ocular surface temperature.**

  Authors: José Luis Hernández- Verdejo**, Laura Rico-del-Viejo,** María García-Montero, Nina Tavberidze, Amalia Lorente-Velázquez, David Madrid-Costa.

  14° International Conference of Optometry and Vision Science (CIOCV'2017). University of Minho, Braga, Portugal. April 22-23th, 2017

- **Impact on anterior ocular surface of three different types of soft contact lenses materials.**

  Authors: Nina Tavberidze, Amalia Lorente-Velázquez, **Laura Rico-del-Viejo,** María García-Montero, José Luis Hernández- Verdejo**,** David Madrid-Costa.

  14° International Conference of Optometry and Vision Science (CIOCV'2017). University of Minho, Braga, Portugal. April 22-23th, 2017

- **Ocular surface temperature in patients with evaporative and aqueous-deficient dry eye relative to normal.**

  Authors: Irene Martínez-Alberquilla, Gabriela Orbezo, Álvaro Carrasco, María García- Montero, Clara Llorens-Quintana, **Laura Rico-del-Viejo.**

  VI International Congress of Research in Retina and Vision. Inst. Investigaciones Oftalmológicas Ramón Castro Viejo, Madrid, Spain. June 29-30th, 2018

- **Comparison of the ocular surface temperature and evaporation among different lipid layer patterns.**

  Authors: Gabriela Orbezo, Álvaro Carrasco, Irene Martínez-Alberquilla, María García- Montero, **Laura Rico-del-Viejo,** Clara Llorens-Quintana.

  VI International Congress of Research in Retina and Vision. Inst. Investigaciones Oftalmológicas Ramón Castro Viejo, Madrid, Spain. June 29-30th, 2018

- **Comparación de los cambios temporales de temperatura de la superficie ocular durante el uso de diferentes tipos de lentes de contacto.**

  Authors: José Luis Hernández-Verdejo, **Laura Rico-del-Viejo**, María García-Montero. Fernando Javier Gómez-Sanz, Nina Tavberidze, Amalia Lorente- Velázquez, David Madrid-Costa.

  25° Congreso Internacional de Optometría, Contactología y Óptica Oftálmica (OPTOM) Madrid, Spain. April 13-15th, 2018

- **Estudio de la dinámica de la película lagrimal en la calidad óptica del ojo durante el parpadeo con diversos materiales de lentes de contacto blandas.**

  Authors: María García-Montero, José Luis Hernández-Verdejo, **Laura Rico-del-Viejo**, Amalia Lorente- Velázquez, Nina Tavberidze, Fernando Javier Gómez-Sanz, Nina Tavberidze, Irene Martínez- Alberquilla, David Madrid-Costa.

  25º Congreso Internacional de Optometría, Contactología y Óptica Oftálmica (OPTOM) Madrid, Spain. April 13-15th, 2018

- **Impacto del porte de lentes de contacto en la sintomatología del usuario.**

  Authors: Fernando Javier Gómez-Sanz, María García-Montero, José Luis Hernández-Verdejo, **Laura Rico-del-Viejo**, Nina Tavberidze, Amalia Lorente- Velázquez, David Madrid-Costa, Irene Martínez-Alberquilla.

  25° Congreso Internacional de Optometría, Contactología y Óptica Oftálmica (OPTOM) Madrid, Spain. April 13-15th, 2018

- **Impacto sobre la superficie ocular de una lente de contacto neofilcon A para dos poblaciones de sujetos: Jóvenes y Présbitas.**

  Authors: Amalia Lorente- Velázquez, María García-Montero, **Laura Rico-del-Viejo**, José Luis Hernández-Verdejo, Nina Tavberidze, Fernando Javier Gómez-Sanz, David Madrid-Costa.

  25° Congreso Internacional de Optometría, Contactología y Óptica Oftálmica (OPTOM) Madrid, Spain. April 13-15th, 2018

- **Automatic and Objective assessment of Meibomian glands structure and dropout.**

  Authors: Clara Llorens-Quintana**, Laura Rico-del-Viejo,** Piotr Syga, Cezary Sielużycki, D. Robert Iskander

  Annual ARVO Meeting. Hawaii Convection Center, Honolulu (Hawaii, EEUU), 29- 3rd May, 2018

- **The impact of two contact lenses designed for visual display terminals on the ocular surface of habitual contact lens wearers.**

  Authors: Irene Martínez- Alberquilla, **Laura Rico-del-Viejo**, Fernando Javier Gómez-Sanz, Amalia Lorente- Velázquez, José Luis Hernández-Verdejo, María García-Montero.

  15° International Conference of Optometry and Vision Science CIOCV'2018. University of Minho, Braga, Portugal. April 28-29th

- **How affect the contact lenses designed for "visual display terminal" in the ocular surface integrity?**

  Authors: Irene Martínez- Alberquilla, **Laura Rico-del-Viejo**, Fernando Javier Gómez-Sanz, Amalia Lorente- Velázquez, José Luis Hernández-Verdejo, María García-Montero.

  15° International Conference of Optometry and Vision Science CIOCV'2018. University of Minho, Braga, Portugal. April 28-29th

- **The relationship between new morphological and objective Meibomian glands parameters and relevant ocular surface parameters.**

  Authors: **Laura Rico-del-Viejo**, Clara Llorens-Quintana, Javier Ruiz-Alcocer, Irene Martínez-Alberquilla, María García-Montero, David Madrid-Costa.

  16° International Conference of Optometry and Vision Science CIOCV'2018. Altice Forum Braga, Braga, Portugal. May 4-5th

Outreach Activities

- Open-house day for the **Erasmus+ Program "Little Scientists"** at the Faculty of Optics and Optometry, Complutense University of Madrid, Spain.

  Participation: Laura Rico-del-Viejo and Duygu Acar.

- **2nd Edition "OPTOMEVISTA" (NEOUM).** University of Minho, Braga, Portugal. May 21st, 2016

  "European Dry Eye Network: The future of Dry Eye Research"

  Authors: Laura Rico-del-Viejo and David Madrid-Costa.

- **IV Jornadas AEOPTOMETRISTAS 2017**. Madrid, Spain. February 11th 2017 *"European Dry Eye Network (EDEN)"*

  Authors: Laura Rico-del-Viejo and David Madrid-Costa.

- Summer Exhibition at the ***Royal Society of Science*** with the Project *"Not a Dry Eye in the house"*.

  Coordinator: Prof. James Wolffsohn (Aston University). London July 2-8th 2018

- Coordinator of the second edition of **"PhDAY 2018"** in the Faculty of Optics and Optometry (November 22nd 2018), Complutense University of Madrid, Madrid, Spain.

Other Activities

- **Collaboration in a Multicenter DED study**

  Prevalence and Risk Factors of DED

  Promotor: Aston University (Birmingham)

  Coordinator: Professor James Wolffsohn

- **Collaboration in Books**

  - **Superficie ocular y lentes de contacto -** Fundación Salud Visual (February 2016)
    - Language: Spanish
    - **Authors:** José M. González Méijome and César Villa Collar.
    - **Collaboration in:** CHAPTER 7
  - **Actualización en Queratocono.**
    - Language: Spanish
    - **Authors:** Cristina Peris Martínez and Nicolás Alejandre Alba
    - **Collaboration in:** CHAPTER 10

- **Reviewer**
  - Contact Lens and Anterior Eye (2017)
  - Journal of Optometry (2016-2018)
  - Journal of Ophthalmology (2018-2019)
- **Industrial sector training**
  - Collaboration in R&D and Marketing Department in Mark'ennovy (Madrid, Spain).
- **Teaching and mentoring**
  - Pediatric Optometry (30 hours)
  - Mentoring and supervision of MSc students.
- **Event management**
  - 2° Edition PhDAY 2018, Faculty of Optics and Optometry (Madrid, Spain). November 22^{sd}, 2018.
- **Other activities related to EDEN:**
  - Collaboration in the EDEN website
  - Collaboration in the editing of EDEN videos

  EDEN training courses:
  - Complutense University of Madrid, Spain
  - Aston University, Birmingham, UK
  - WUST, Wroclaw, Poland
  - Mid-term meeting with EU Commitee, Madrid

ANNEX

1. APPROVAL FROM THE ETHICS COMMITTEE

Hospital Clínico San Carlos

Informe Dictamen Protocolo Favorable

C.P. Horizon 2020 GA number-642760) - C.I. 16/279-E_BS

16 de junio de 2016

Dra. Mar García Arenillas
Presidenta del CEIC Hospital Clínico San Carlos

CERTIFICA

Que el CEIC Hospital Clínico San Carlos en su reunión del día 08/06/2016, acta 6.1/16 ha evaluado la propuesta del promotor/investigador referida al estudio:

Título: ***"InfraredImaging of Meibomian GlandStructure"***

Que en este estudio:

- Se cumplen los requisitos necesarios de idoneidad del protocolo en relación con los objetivos del estudio y están justificados los riesgos y molestias previsibles para el sujeto.
- Es adecuado el procedimiento para obtener el consentimiento informado.
- La capacidad del investigador y los medios disponibles son adecuados para llevar a cabo el estudio.
- El alcance de las compensaciones económicas previstas no interfiere con el respeto de los postulados éticos.
- Se cumplen los preceptos éticos formulados en la Declaración de Helsinki de la Asociación Médica mundial sobre principios éticos para las investigaciones médicas en seres humanos y en sus posteriores revisiones, así como aquellos exigidos por la normativa legal aplicable en función de las características del estudio.

Es por ello que el Comité **informa favorablemente** sobre la realización de dicho proyecto por el **Dr. David Madrid Costa** y la **Dra. Laura Rico del Viejo** como investigadores en la Facultad de Óptica y Optometría de la Universidad Complutense de Madrid.

Lo que firmo en Madrid, a 16 de junio de 2016

Dra. Mar García Arenillas
Presidenta del CEIC Hospital Clínico San Carlos

2. PARTICIPANT INFORMATION SHEET AND INFORMED CONSENT

HOJA DE INFORMACIÓN AL PARTICIPANTE

Título del Estudio: "Evaluación de la estructura de las glándulas de meibomio mediante tecnología infrarroja"

Título del Estudio (original): "Infrared Imaging of Meibomian Gland Structure"

INVESTIGADORES: José Manuel Benítez del Castillo[1]
David Madrid Costa[2]
Laura Rico del Viejo[2]

1. Unidad de Superficie Ocular (Oftalmología) del Hospital Universitario Clínico San Carlos, Madrid.
2. Departamento de Óptica II: Optometría y Visión, Facultad de Óptica y Optometría, UCM

LOCALIZACIÓN DEL ESTUDIO: Facultad de Óptica y Optometría
C. Arcos de Jalón Nº 118
28037 MADRID

INTRODUCCIÓN

Estimado Paciente,

Se le invita a participar en un estudio de investigación clínica cuyo objetivo principal es *actualizar la clasificación de la disfunción de las glándulas de meibomio (DGM) basada en la estructura de las glándulas revelada por técnicas de imagen*.

Antes de decidirse a participar en este estudio, es importante que entienda por qué se realiza el estudio y lo que implicará. Por favor, tómese su tiempo para leer con detenimiento la siguiente información y pregunte al responsable del estudio si hay algo que no está claro o si desea más información.

Este estudio cumple con la legislación vigente: Declaración de Helsinki, Convenio de Oviedo, Ley 274/2002 de autonomía del paciente, Ley orgánica de protección de datos 15/1999 ***(consultar artículos sobre derechos de acceso, rectificación, cancelación y oposición explicados en el anexo de este documento)*** y Ley 14/2007 de investigación biomédica.

Su participación en este estudio es totalmente voluntaria y puede decidir no participar o cambiar su decisión en cualquier momento, sin que por ello se produzca perjuicio alguno. Del mismo modo, puede esperar a consultar con otra profesional antes de tomar la decisión de participar en el estudio. La participación en el estudio no le supondrá coste alguno. No hay remuneración económica para usted ni para los investigadores. La recogida de datos para este estudio no implica ningún riesgo para usted. La información recogida será únicamente empleada para investigación clínica.

Todos sus datos serán reconocidos mediante un código y sólo los investigadores podrán relacionar dichos datos con usted. Conforme a las disposiciones legales, usted tiene derecho a conocer los datos del estudio que sobre usted se recojan, a obtener una copia, y a conocer el uso que puedan darse a los mismos. Dicha información le será suministrada por el investigador. En el caso de que con los datos del estudio se realizará alguna publicación científica su identidad será totalmente confidencial.

Si posteriormente desea hacer alguna pregunta o revocar el consentimiento, se pondrá en contacto con los investigadores:

- Dr. David Madrid Costa correo electrónico: damadrid@ucm.es
- Laura Rico del Viejo correo electrónico: larico@ucm.es

DESCRIPCIÓN DEL ESTUDIO

Los lípidos producidos por las glándulas de meibomio son el componente principal de la capa lipídica de la película lagrimal. Es sabido que la capa lipídica protege contra la evaporación de la fase acuosa, proporciona lubricación y también ayuda a estabilizar la película lagrimal. Por esta razón, los lípidos secretados por las glándulas de meibomio son esenciales para el mantenimiento de la superficie ocular[147].La disfunción de las glándulas de Meibomio (DGM) es la anomalía más común en la práctica oftalmológica[338] y la principal causa de ojo seco. En 2011, *Tear Film and Ocular Surface Society* (TFOS) definió la DGM como una anomalía crónica de las glándulas de meibomio que se caracteriza por la obstrucción del conducto terminal o cambios cualitativos o cuantitativos en la secreción glandular[158]. Esto puede resultar en una alteración de la película lagrimal, síntomas de irritación ocular, inflamación clínicamente aparente y enfermedad de la superficie ocular[158]. A pesar de que esta condición influye en la salud y en el bienestar de millones de personas, no existe un consenso general respecto a la definición, clasificación, diagnóstico o terapia de la DGM. Gran parte de este desconocimiento se debe a la ausencia de un método de observación clínico que sea práctico para el diagnóstico ocular.

La Meibografía de no contacto es una tecnología reciente que proporciona la evaluación "in vivo" de la morfología de las glándulas y ha demostrado ser útil en el diagnóstico de la DGM[169,178] Esta tecnología utiliza luz infrarroja (IR) para trans-iluminar el párpado y con la ayuda de una cámara CCD registra imágenes de las glándulas de meibomio de ambos párpados superior e inferior[157]. La pérdida de tejido glandular (comúnmente denominado *dropout*) se refiere a la pérdida parcial o total de las glándulas[166]. Esta pérdida aumenta con la edad en sujetos normales, pero no necesariamente debido a la presencia de DGM obstructiva[339]. Se ha postulado que la creciente evaporación de la capa acuosa de la película lagrimal está relacionada con la extensión de pérdida glandular existente[340].

Otra tecnología utilizada para evaluar las glándulas de meibomio es la Microscopía Confocal Láser. Esta herramienta no invasiva permite obtener imágenes de alta

resolución de los tejidos vivos a nivel celular, proporcionando imágenes comparables a los métodos histoquímicos[341]. Particularmente aplicado a la observación de las glándulas, proporciona información sobre la densidad y el diámetro de los acinos, la reflectividad de la secreción, la inflamación periglandula y las características de orificio.

Los cambios producidos en las distintas estructuras de las glándulas en pacientes de ojo seco y otras condiciones oculares han sido observados en varios estudios científicos con esta tecnología[89,198]

OBJETIVO DEL ESTUDIO

El objetivo de este estudio es la actualización de la clasificación de la DGM basada en la estructura de las glándulas de meibomio revelada por técnicas de imagen. Las tecnologías antes mencionadas se utilizarán para identificar los cambios en la estructura de las glándulas de meibomio que nos ayudan a predecir la evolución de esta disfunción.

BENEFICIOS DEL ESTUDIO

La participación en este estudio puede aportar al voluntario información valiosa sobre el estado de su superficie ocular y la obtención de recomendaciones personalizadas por parte de los profesionales de la visión que trabajan en este proyecto para mejorar su salud ocular. La información que se obtendrá con este estudio será de gran relevancia para los médicos oftalmólogos ya que la clasificación que se pretende realizar ayudará al clínico a realizar un mejor diagnóstico de la disfunción de las glándulas de meibomio, siendo esta la anomalía más frecuente en las consultas de oftalmología de todo el mundo.

DURACIÓN TOTAL DE SU PARTICIPACIÓN

La duración esperada del estudio es de 1 día.

LOS EXÁMENES CLÍNICOS

Durante el estudio, el responsable del estudio le realizará una serie de exámenes que se describirán a continuación. Los exámenes que se realizarán en el marco del estudio serán de dos tipos: aquéllos que se basen en determinar su función ocular (como funciona su vista y que signos o síntomas presenta) y los exámenes relacionados con la observación y medida de la morfología de las glándulas de meibomio.

Primero se realizará una anamnesis en profundidad a cada participante en la que se incluirán preguntas relevantes para el estudio y para los criterios de inclusión del mismo. A continuación se les realizará un cuestionario subjetivo para cuantificar la posible sintomatología asociada con el ojo seco.

Después de esta primera parte, se procederá a realizar toda la batería de exámenes clínicos.

1º Evaluación de la sintomatología de ojo seco.

La evaluación de la sintomatología juega un papel fundamental en el diagnóstico clínico del ojo seco. Por este motivo, los participantes tendrán que rellenar varios cuestionarios sobre ojo seco validados para conocer la sintomatología en cada caso.

2º Osmolaridad de la película lagrima.

La evaluación de la osmolaridad lagrimal es especialmente importante ya que actualmente es posiblemente la medida objetiva más fiable para diagnosticar el ojo seco. Se procederá a la evaluación de la osmolaridad lagrimal de cada ojo del paciente utilizando un dispositivo para dicho propósito.

3º Evaluación de la superficie ocular con el dispositivo Keratograph 5M.

Conocer el estado de la integridad de la superficie ocular es importante a la hora de determinar la gravedad del ojo seco. Para ello, nuevos dispositivos han sido desarrollados para realizar una evaluación no invasiva de todos los componentes importantes de la superficie ocular. Se procederá a evaluar los parámetros de la superficie ocular con el Keratograph 5M, un topógrafo corneal avanzado.

4º Evaluación de la temperatura de la superficie ocular mediante termografía infrarroja.

La termografía infrarroja es una tecnología que ha cobrado mucha importancia en el campo de la oftalmología. Se procederá al registro de la temperatura ocular del participante mediante una cámara térmica infrarroja. Esta evaluación es no invasiva y se realizará en condiciones naturales de parpadeo del participante.

5º Evaluación de las estructuras de las superficie ocular con lámpara de hendidura.

Como se ha mencionado anteriormente, conocer el estado de la integridad de la superficie ocular es importante a la hora de determinar la gravedad del ojo seco. Por ello, la evaluación exhaustiva de las estructuras se realizará mediante lámpara de hendidura. También se procederá a aplicar fluoresceína y verde lisamina (tintes oculares) para evaluar el estado de los tejidos oculares sin ningún tipo de repercusión para la visión del participante.

6º Evaluación de la morfología de las glándulas de meibomio mediante meibografía infrarroja.

La integridad de las glándulas de meibomio es importante para mantener la homeostasis de la superficie ocular ya que de ellas dependen la producción de los lípidos que forman parte de la capa lipídica de la película lagrimal. Por este motivo, se procederá a realizar

meibografía de no contacto para la observación de la morfología de las glándulas de meibomio. También se graduarán con escalas subjetivas la expresibilidad de las glándulas, la calidad de la secreción y la tortuosidad que presentan las glándulas.

7º Evaluación de la producción lagrimal mediante el test de Schirmer.

La evaluación de la secreción lagrimal nos aporta información sobre la producción acuosa.
Este test consisten en la inserción de una tira de papel en el borde temporal del párpado inferior. Tras su inserción, se deberá medir la longitud de tira o hilo mojada por la película lagrimal en un determinado periodo de tiempo.

ANEXO

Ley orgánica de protección de datos 15/1999:

- **Artículo 15: Derechos de acceso**

1. El interesado tendrá derecho a solicitar y obtener gratuitamente información de sus datos de carácter personal sometidos a tratamiento, el origen de dichos datos, así como las comunicaciones realizadas o que se prevén hacer de los mismos.

2. La información podrá obtenerse mediante la mera consulta de los datos por medio de su visualización, o la indicación de los datos que son objeto de tratamiento mediante escrito, copia, telecopia o fotocopia, certificada o no, en forma legible e inteligible, sin utilizar claves o códigos que requieran el uso de dispositivos mecánicos específicos.

3. El derecho de acceso a que se refiere este artículo sólo podrá ser ejercitado a intervalos no inferiores a doce meses, salvo que el interesado acredite un interés legítimo al efecto, en cuyo caso podrán ejercitarlo antes.

- **Artículo 16: Derecho de rectificación y cancelación**

1. El responsable del tratamiento tendrá la obligación de hacer efectivo el derecho de rectificación o cancelación del interesado en el plazo de diez días.

2. Serán rectificados o cancelados, en su caso, los datos de carácter personal cuyo tratamiento no se ajuste a lo dispuesto en la presente Ley y, en particular, cuando tales datos resulten inexactos o incompletos.

3. La cancelación dará lugar al bloqueo de los datos, conservándose únicamente a disposición de las Administraciones públicas, Jueces y Tribunales, para la atención de las posibles responsabilidades nacidas del tratamiento, durante el plazo de prescripción de éstas. Cumplido el citado plazo deberá procederse a la supresión.

4. Si los datos rectificados o cancelados hubieran sido comunicados previamente, el responsable del tratamiento deberá notificar la rectificación o cancelación efectuada a quien se hayan comunicado, en el caso de que se mantenga el tratamiento por este último, que deberá también proceder a la cancelación.

5. Los datos de carácter personal deberán ser conservados durante los plazos previstos en las disposiciones aplicables o, en su caso, en las relaciones contractuales entre la persona o entidad responsable del tratamiento y el interesado.

Artículo 17: Procedimiento de oposición, acceso, rectificación o cancelación

1. Los procedimientos para ejercitar el derecho de oposición, acceso, así como los de rectificación y cancelación serán establecidos reglamentariamente.

2. No se exigirá contra prestación alguna por el ejercicio de los derechos de oposición, acceso, rectificación o cancelación.

UNIVERSIDAD COMPLUTENSE MADRID

CONSENTIMIENTO INFORMADO

(COPIA PARA INVESTIGADOR)

Código de identificación del paciente: _____

Título del Estudio: *"Evaluación de la estructura de las glándulas de meibomio mediante tecnología infrarroja"*

INVESTIGADORES David Madrid Costa[1]
Laura Rico del Viejo[1]
José Manuel Benítez del Castillo[2]

1.Departamento de Óptica II: Optometría y Visión, Facultad de Óptica y Optometría, UCM
2. Unidad de Superficie Ocular (Oftalmología) del Hospital Universitario Clínico San Carlos, Madrid.

Introducción

LOCALIZACIÓN DEL ESTUDIO Facultad de Óptica y Optometría
Calle Arcos de Jalón Nº 118
CP 28037 MADRID

ca sus iniciales en los recuadros ↓

1. Confirmo que he leído y entendido la información del presente estudio dada el día …… y que he tenido oportunidad de realizar preguntas sobre el mismo. ☐
2. Comprendo que mi participación en el estudio es voluntaria y que este no niega la necesidad de un examen ocular periódico. ☐
3. Comprendo que puedo retirarme del estudio cuando quiera y sin repercusión a mis derechos legales. ☐
4. Presto libremente mi conformidad para participar en el estudio. ☐

_____	_____	_____
Nombre del participante	Fecha	Firma
_____	_____	_____
Nombre del investigador	Fecha	Firma

UNIVERSIDAD COMPLUTENSE MADRID

CONSENTIMIENTO INFORMADO

(COPIA PARA PARTICIPANTE)

Código de identificación del paciente: _____

Título del Estudio: *"Evaluación de la estructura de las glándulas de meibomio mediante tecnología infrarroja"*

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LOCALIZACIÓN DEL ESTUDIO Facultad de Óptica y Optometría
Calle Arcos de Jalón Nº 118
CP 28037 MADRID

Introduzca sus iniciales en los recuadros ↓

1. Confirmo que he leído y entendido la información del presente estudio dada el día …… y que he tenido oportunidad de realizar preguntas sobre el mismo. ☐
2. Comprendo que mi participación en el estudio es voluntaria y que este no niega la necesidad de un examen ocular periódico. ☐
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4. Presto libremente mi conformidad para participar en el estudio. ☐

_____	_____	_____
Nombre del participante	Fecha	Firma
_____	_____	_____
Nombre del investigador	Fecha	Firma

3. CLINICAL SHEET

Ficha Clínica

Fecha:
Hora: Paciente:

Nombre y Apellidos: _____
Sexo: M☐ F☐
Fecha de nacimiento:_____ Edad: _____

Profesión: _____

- **¿Consentimiento informado? Si ☐**

Anamnesis

Salud general (tache lo que proceda)

1. Tiene o ha padecido:
   ☐ anemia, ☐ diabetes, ☐ infecciones frecuentes de oído o garganta, ☐ sinusitis, ☐ artritis, ☐ reumatismo, ☐ trastorno de la tiroides, ☐ hipertensión arterial, ☐ enfermedades de la piel, ☐ enfermedades del colágeno, ☐ asma, ☐ bronquitis, ☐enfisema
2. ¿Es fumador? SI☐ NO☐ Número de cigarrillos al día: ____
3. ¿Padece alguna alergia? ☐SI ☐NO ¿Cuál?_____
4. ¿Toma normalmente o está tomando alguna medicación? (aunque no sea para los ojos)
   ☐SI ☐NO ¿Cuál? ¿Para qué? ¿Desde cuándo?
5. ¿Usa lágrimas artificiales? ☐SI ☐NO ¿Cuántas veces al día? ____
   ¿A qué hora se ha puesto la última gota? _____
6. Si es mujer, ¿está tomando anticonceptivos orales? ☐SI ☐NO
   ¿Desde cuándo? _____
7. ¿Está embarazada? ☐SI ☐NO

Historial Visual

8. ¿A qué edad empezó a usar gafas/ LC? Última revisión:
9. ¿Ha tenido o tiene alguno de los siguientes procesos oculares? (tache lo que proceda)

Ojo Derecho Ojo Izquierdo ¿Hace cuánto tiempo?

Ojo vago	☐	☐	.....
Estrabismo	☐	☐	.....
Enfermedad de retina	☐	☐	.....
Úlcera corneal	☐	☐	.....
Conjuntivitis frecuentes	☐	☐	.....
Defecto epitelial recurrente	☐	☐	.....
Queratocono	☐	☐	.....
Ojo seco severo	☐	☐	.....
Enfermedad superficie ocular	☐	☐	.....
Glaucoma	☐	☐	.....
Cataratas	☐	☐	.....
Cirugía ocular (especificar)	☐	☐	.....
Otros (especificar)	☐	☐	.....

10. ¿Alguien de su familia ha tenido alguno de los procesos oculares anteriormente descritos? (especificar)

Tratamientos (pasados y presentes)

Información adicional a tener en cuenta:

Pruebas Clínicas

❖ Osmolaridad de la película lagrimal

OD ☐☐☐ **OI** ☐☐☐

<u>Ojo escogido</u>: OD / OI Motivo: ☐

❖ Parámetros del Keratograph

<u>Altura del menisco lagrimal (x3)</u>

1. ☐.☐☐**mm**
2. ☐.☐☐**mm**
3. ☐.☐☐**mm**

<u>Enrojecimiento ocular</u> (x3)

Bulbar

1. **Temporal** ☐.☐ **Nasal** ☐.☐

Limbal

Temporal ☐.☐ **Nasal** ☐.☐

Bulbar

2. **Temporal** ☐.☐ **Nasal** ☐.☐

Limbal

Temporal ☐.☐ **Nasal** ☐.☐

Bulbar

3. **Temporal** ☐.☐ **Nasal** ☐.☐

Limbal

Temporal ☐.☐ **Nasal** ☐.☐

<u>Capa lipídica (vídeo) (x1)</u> ☐

Viscosidad de la película lagrimal (vídeo) (x1) ☐

NIKBUT (x3)

FR:

1)☐☐.☐☐ **2)**☐☐☐☐ **3)**☐☐☐☐

AVG:

1)☐☐.☐☐ **2)**☐☐☐☐ **3)**☐☐☐☐

❖ Evaluación de la temperatura de la superficie ocular

- Párpados
- Córnea y Conjuntiva
- Dinámica de la película lagrimal (registro de 40 segundos -parpadeo normal)

❖ Evaluación de la superficie ocular (Lámpara de Hendidura)

- CÓRNEA, CONJUNTIVA y PÁRPADOS (Luz Difusa) (Evaluación General)

Observaciones:_____
_____
_____

- PÁRPADOS

Borde del párpado

Grosor del borde libre 0-5 _____

Irregularidad del margen 0-1 _____

Telangiectasias 0-1 _____

Distiquiasis 0-1 _____

Madarosis 0-1 _____

Malposición 0-1 _____

Unión cutaneomucosa (línea de Marx): posicionamiento posterior

(0-3) _____

Orificios glandulares

Pouting: sobreelevación del orificio 0-1 _____

Capping: cúpula grasa queratinizada sobre el orificio 0-1 _____

Pérdida de definición de bordes 0-1 _____

Invasión vascular 0-1 _____

Estrechamiento del orificio 0-1 _____

Posicionamiento posterior a la línea de Marx 0-1 _____

Acinos glandulares

Facilidad de visualización 0-3 _____

Dilataciones quísticas 0-3 _____

Concreciones 0-3 _____

Chalazión 0-3 _____

Secreción

Espumosa 0-1 _____

Volumen

Calidad 0-3 _____

Facilidad de expresión 0-3 _____

Ausencia de secreción 0-1 _____

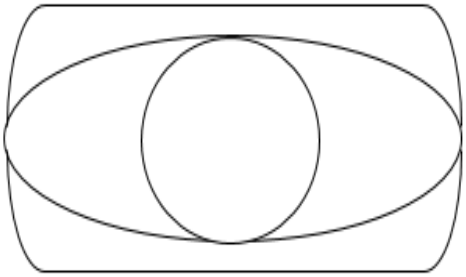
Observaciones:_____

_____

_____

- TINCIÓN CORNEAL (Fluoresceína)
  - Escala **OXFORD**
    (Evaluar tinción a los 2 minutos de la instilación de fluoresceína)

**Hacemos TBUT durante ese tiempo*



(Escala Oxford)

OD	**□0**	**□1**	**□2**	**□3**	**□4**
OI	**□0**	**□1**	**□2**	**□3**	**□4**

- **TBUT**
  1. **_____s**
  2. **_____s**
  3. **_____s**

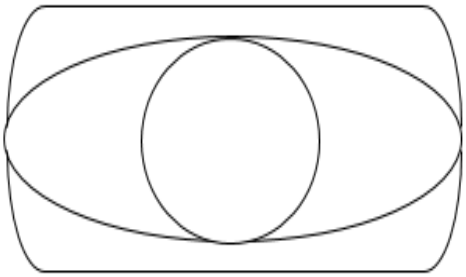
Observaciones:

_____
_____
_____

*REGISTRO EN FOTOS *(KERATOGRAPH)* DE FLUORESCEÍNA (POSICIÓN PRIMARIA DE MIRADA, OTRAS POSICIONES Y BORDE PÁRPADO INFERIOR)

- TINCIÓN VERDE LISAMINA

Escala **OXFORD**



(Escala Oxford)

OD	**□0**	**□1**	**□2**	**□3**	**□4**
OI	**□0**	**□1**	**□2**	**□3**	**□4**

Observaciones:

_____

*REGISTRO EN FOTOS *(KERATOGRAPH)* VERDE LISAMINA (POSICIÓN PRIMARIA DE MIRADA, OTRAS POSICIONES Y LID WIPER SUPERIOR E INFERIOR)

- ✓ Limpiados el ojo con solución salina.

*Esperamos 10 minutos para dejar recuperar (Tiempo para cuestionarios subjetivos en el caso de que no se hayan completado anteriormente).

❖ **Meibografía Infrarroja**

Párpado superior ☐ Párpado inferior ☐

DROPOUT (0-3): _____

Observaciones:_____

❖ **Test de Schirmer (5 minutos con anestésico tópico).** ☐SI ☐NO

Volumen: _____

Observaciones:_____

_____

: The Ocular-surface-disease-index (OSDI)[72], Mcmonnies (MQ)[83], the standard patient evaluation of eye dryness (SPEED)[82], the Symptom Assessment in Dry Eye (SANDE)[76] and the Dry Eye Questionnaire (DEQ-5; short version)[78].

4. DED QUESTIONNAIRES

1. The Ocular Surface Disease Index (OSDI)

UNIVERSIDAD COMPLUTENSE MADRID

Hospital Universitario Clínico San Carlos
SaludMadrid
Comunidad de Madrid

OCULAR SURFACE DISEASE INDEX®

El Test OSDI (ocular surface disease index) es un test sencillo creado para establecer una gravedad y clasificación del ojo seco según su sintomatología.

Apellidos y Nombre: _____, _____

Fecha: _____/_____/_____ Código y Grupo:_____

Conteste a las siguientes preguntas marcando la casilla que mejor represente su respuesta.

¿Ha experimentado alguna de las siguientes alteraciones <u>durante la última semana?</u>

	En todo momento	Casi en todo momento	El 50% del tiempo	Casi en ningún momento	En ningún momento
1. Sensibilidad a la luz					
2. Sensación de arenilla en los ojos					
3. Dolor de ojos					
4. Visión borrosa					
5. Mala visión					

¿Ha tenido problemas en los ojos que le han limitado o impedido realizar alguna de las siguientes actividades <u>durante la última semana?</u>

	En todo momento	Casi en todo momento	El 50% del tiempo	Casi en ningún momento	En ningún momento	N/A
6. Leer						
7. Conducir de noche						
8. Trabajar con un ordenador o utilizar un cajero automático						
9. Ver la televisión						

¿Ha sentido incomodidad en los ojos en alguna de las siguientes situaciones <u>durante la última semana?</u>

	En todo momento	Casi en todo momento	El 50% del tiempo	Casi en ningún momento	En ningún momento	N/A
10. Viento						
11. Lugares con baja humedad (muy secos)						
12. Zonas con aire acondicionado						

2. Symptom Assessment in Dry Eye (SANDE)

CUESTIONARIO SANDE

Por favor, complete las siguientes preguntas sobre la frecuencia y la severidad de los síntomas del ojo seco:

1. **<u>FRECUENCIA</u> de los síntomas:**

   Por favor, coloque una **X** sobre la línea indicando como es de frecuente, en promedio, siente sus **ojos secos y/o irritados**:

   _____

   Raramente **Todo el tiempo**

2. **<u>SEVERIDAD</u> de los síntomas:**

   Por favor, coloque una **X** sobre la línea indicando como es de severo, en promedio, los síntomas de **sequedad y/o irritación**:

   _____

   Muy suave **Muy severo**

3. Standard Patient Evaluation of Eye Dryness (SPEED)

UNIVERSIDAD COMPLUTENSE MADRID

Hospital Universitario Clínico San Carlos
SaludMadrid
Comunidad de Madrid

CUESTIONARIO SPEED II

Apellidos y Nombre: _____, _____
Fecha: _____/_____/_____ Fecha de nacimiento: _____/_____/_____
Código y Grupo:_____

El síndrome de ojo seco es la razón más frecuente por la que los pacientes visitan al oftalmólogo. Por lo tanto, pedimos que se tome un momento y complete cuidadosamente el siguiente cuestionario:

Indique la **FRECUENCIA** de los síntomas que usted experimenta marcando *nunca, a veces, a menudo o constante* a continuación:

0 = Nunca , 1 = A veces , 2 = A menudo , 3 = Constante

Síntomas	0	1	2	3
Sequedad, Sensación de arenilla o Picazón				
Dolor o Irritación				
Ardor y Lagrimeo				
Fatiga Ocular				

Indique la **SEVERIDAD** de los síntomas que usted experimenta usando la lista que se presenta a continuación:

0= No tengo problemas
1= Tolerable- No es perfecto pero no resulta incómodo
2= Incómodo- Es irritante pero no interfiere en mi día a día
3= Molesto- Es irritante e interfiere en mi día a día
4= Intolerable- Incapaz de realizar mis tareas diarias

Síntomas	0	1	2	3	4
Sequedad, Sensación de arenilla o Picazón					
Dolor o Irritación					
Ardor y Lagrimeo					
Fatiga Ocular					

Por favor, marque con una X si ha experimentado estos síntomas:

1. Hoy_____
2. En las últimas 72 horas_____
3. En los pasados 3 meses_____

- ¿Utiliza lágrimas artificiales o pomada? SI / NO

  En caso afirmativo, ¿Cual utiliza?_____

- ¿Le han dicho que tiene blefaritis o ha sido tratado por un orzuelo?

  Blefaritis: SI/NO
  Orzuelo: SI/NO

- ¿Ha experimentado fluctuación de la visión? (Que puede mejorar con el parpadeo)

  1. Nunca_____
  2. A veces____
  3. Frecuentemente____
  4. Mucho/Siempre_____

Puntuación Total (Frecuencia + Severidad) **=**_____

4. McMonnies (MQ)

UNIVERSIDAD COMPLUTENSE MADRID

Hospital Universitario Clínico San Carlos
SaludMadrid
Comunidad de Madrid

Cuestionario de evaluación de ojo seco de McMonnies

Apellidos y Nombre: _____, _____

Fecha: _____/_____/_____ **Código y Grupo**:_____

Responda a las siguientes preguntas subrayando las respuestas que le parezcan más apropiadas:

Mujer / Hombre.

Edad: menos de 25 años / 25–45 años / más de 45 años.

Actualmente: no llevo lentes de contacto / lentes de contacto rígidas / lentes de contacto blandas.

1. ¿Le han recetado alguna vez un colirio u otro tratamiento para ojo seco?

Sí / No / No lo sé

2. ¿En algún momento ha experimentado alguno de los siguientes síntomas oculares? (subraye los que se le apliquen).

Dolor / Picor / Sequedad / Arenilla / Escozor

3. ¿Con qué frecuencia experimenta estos síntomas? (subrayar)

Nunca / A veces / A menudo/ Constantemente

4. ¿Son sus ojos inusitadamente sensibles al humo del tabaco, la contaminación, el aire acondicionado o la calefacción central?

Sí / No / A veces

5. ¿Sus ojos se ponen muy rojos e irritados al nadar?

No aplicable / Sí / No / A veces

6. ¿Se le secan e irritan los ojos después de beber alcohol?

No aplicable / Sí / No / A veces

7. **¿Toma <u>(subraye, por favor)</u>** comprimidos de antihistamínicos o utiliza colirio antihistamínico, diuréticos (comprimidos fluidos), píldoras para dormir, tranquilizantes, anticonceptivos orales, medicación para la úlcera duodenal, problemas digestivos, alta tensión , antidepresivos o ...?

<u>(escriba cualquier medicación que esté tomando y no aparezca en la lista).</u>

_____

8. ¿Padece artritis? Sí / No / No lo sé

9. ¿Experimenta sequedad de nariz, boca, garganta, pecho o vagina? Nunca / A veces / A menudo / Constantemente

10. ¿Padece alteraciones tiroideas? Sí / No / No lo sé

11. ¿Sabe que duerme con los ojos parcialmente abiertos? Sí / No / A veces

12. ¿Se levanta con los ojos irritados después de dormir? Sí / No / A veces

Puntuaciones: Normal (< 10) Ojo seco marginal (10–20) Ojo seco patológico (>20)

5. Dry Eye Questionnaire (DEQ-5; short version)

1 Preguntas sobre DISCONFORT OCULAR:						
a. Durante un día normal el pasado mes, ¿Con qué frecuencia sintió disconfort ocular?	**0 Nunca**	**1 Raramente**	**2 Algunas veces**	**3 Frecuentemente**	**4 Constantemente**	
b. Cuando sus ojos sienten disconfort, **¿Cómo de intensa fue** esta sensación al final del día, dos horas antes de ir a dormir?	**Nunca**	**En absoluto Intensidad**			**Mucho Intensidad**	
	0	**1**	**2**	**3**	**4**	**5**
2 Preguntas sobre SEQUEDAD OCULAR:						
a. Durante un día normal el pasado mes, ¿Con qué frecuencia sintió sus ojos secos?	**0 Nunca**	**1 Raramente**	**2 Algunas veces**	**3 Frecuentemente**	**4 Constantemente**	
b. Cuando sus ojos sienten disconfort, ¿**Cómo de intensa fue** esta sensación de sequedad al final del día, dos horas antes de ir a dormir?	**Nunca**	**En absoluto Intensidad**			**Mucho Intensidad**	
	0	**1**	**2**	**3**	**4**	**5**
3 Preguntas sobre LAGRIMEO:						
Durante un día normal el pasado mes, ¿Con qué frecuencia sintió sus ojos secos?	**0 Nunca**	**1 Raramente**	**2 Algunas veces**	**3 Frecuentemente**	**4 Constantemente**	
4 Preguntas sobre IRRITACIÓN OCULAR:						
.Durante un día normal el pasado mes, ¿Con qué frecuencia sintió sus ojos irritados?	**0 Nunca**	**1 Raramente**	**2 Algunas veces**	**3 Frecuentemente**	**4 Constantemente**	

5. PERSONAL IMAGES PERMISSIONS

Permiso para uso de imágenes

Yo, JOSÉ ENRIQUE MÉNDEZ MERA, con DNI 02719977C doy permiso a LAURA RICO DEL VIEJO , con DNI 80063778L para utilizar las imágenes en su tesis doctoral, siendo solo para uso privado y académico.

Firmado,

Fecha: 17/10/2018

Yo, Alejandro Martínez Águila, con DNI 46888086X, autorizo a Laura Rico del Viejo para usar en su tesis doctoral las fotos obtenidas durante las pruebas que me realizaron.

Y para que conste, firmo la presente

Madrid, a 17 de octubre de 2018

Permiso para uso de imágenes

Yo, IRENE MARTÍNEZ ALBERQUILLA , con DNI 53990797E doy permiso a LAURA RICO DEL VIEJO , con DNI 80063778L para utilizar las imágenes en su tesis doctoral, siendo solo para uso privado y académico.

Firmado,

Fecha: 17/10/2018

AUTOMATIC AND OBJECTIVE ASSESSMENT OF MEIBOMIAN GLANDS STRUCTURE AND DROP OUT

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1700 – A0221

INTRODUCTION

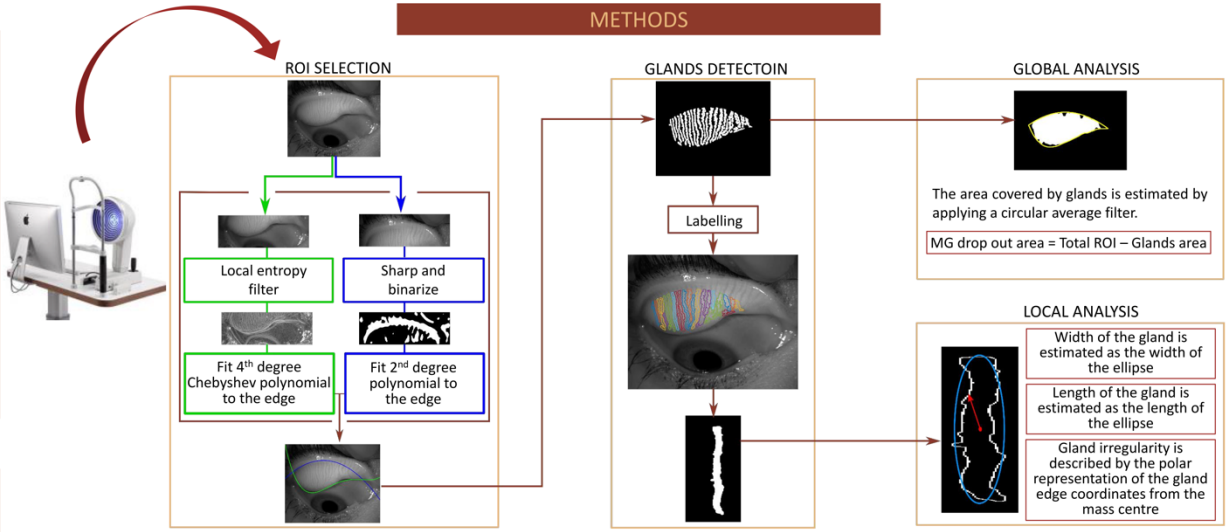
Meibomian Glands (MG) Dysfunction is a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion.[1] The function of MG can be assessed directly by observing their morphology using infrared imaging. However, the assessment of meibography images is still performed subjectively and manually or in the best case semi-automatically, resulting in an increase in diagnostic time and decrease in measurement repeatability. This evaluation consists of subjectively estimating the area of the whole tarsal conjunctiva that is not covered by MG (i.e., MG drop out area). There have been few proposed fully automated algorithms to assess meibography images untill now. However the area of analysis was fixed[2], or they were only able to differentiate between healthy/unhealthy.[3]

We aim at a more versatile and robust approach to analyse MG globally and locally. Developing an algorithm that could work in the majority of cases is a challenging task since infrared meibography images have often low contrast, non-uniform illumination, specular reflections, defocused areas and presence of artifacts. We propose an automated algorithm for morphometric analysis of MG that is reliable and robust to the aforementioned artefacts.

PURPOSE

To develop a new automated and objective methodology for assessing infrared meibography images in order to characterize meibomian glands structure and estimate meibomian gland drop out.

METHODS



RESULTS

Drop out area

According with the meiboscore proposed by Arita et al.[4] to estimate the percentage of meibomian glands drop out area, **objective and automated grades** (range from 0 to 3),were assigned to each image:

- Grade 0: 0% drop out area → 1 subject
- Grade 1: from 1% to 33% drop out area → 5 subjects
- Grade 2: from 34% to 66% drop out area → 3 subjects
- Grade 3: drop out area > 67% → 1 subject

Table 1. Percentage of the area of gland drop out, mean gland length and mean gland width for the four Meiboscore grades determined with the proposed algorithm

Objective Meiboscore	Drop out area (%)	Glands length (mean ± sd) mm	Glands width (mean ± sd) mm
0	0	4	0.5
1	18	3.4 ± 0.7	0.6 ± 0.1
2	41	2.8 ± 1.1	0.5 ± 0.1
3	67	1.1	0.3

MG structure analysis

In order to achieve a better characterization of MG **other parameters besides the MG drop out area** can be extracted using the proposed methodology. Thus, contributing more **information and understanding about structural changes of MG** in MG dysfunction (see Table 1 and Figure 1). These are:

- **Mean gland length**
- **Mean gland width**
- **Irregularity of the glands**

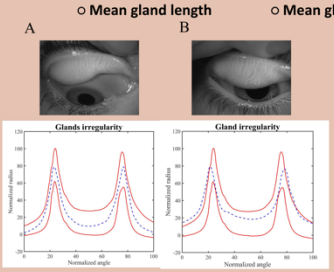


Figure 1. Example of the **irregularity measurement** for an eyelid with regular (left) and tortuous glands (right). Red lines represent the limits for a regular gland computed as the mean radial values ± one standard deviation of the polar coordinates of the edge from 200 healthy glands. Blue dashed lines represent the mean radial values of the polar coordinates of all the detected glands in the studied eyelid. The amount of irregularity is proportional to the amount of area enclosed between red lines and blue dashed lines out of the limits.

CONCLUSIONS

The proposed method is able to **grade the drop out area in an automated and objective way**. In addition, it provides **information on the structure of the glands** (length, width and irregularity) which may be useful in the meibomian glands dysfunction diagnostics and understanding.

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